10 June 2005

Science

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Pages 1501–1696 $10

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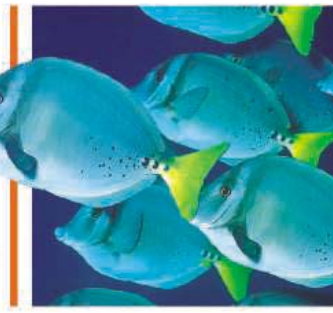



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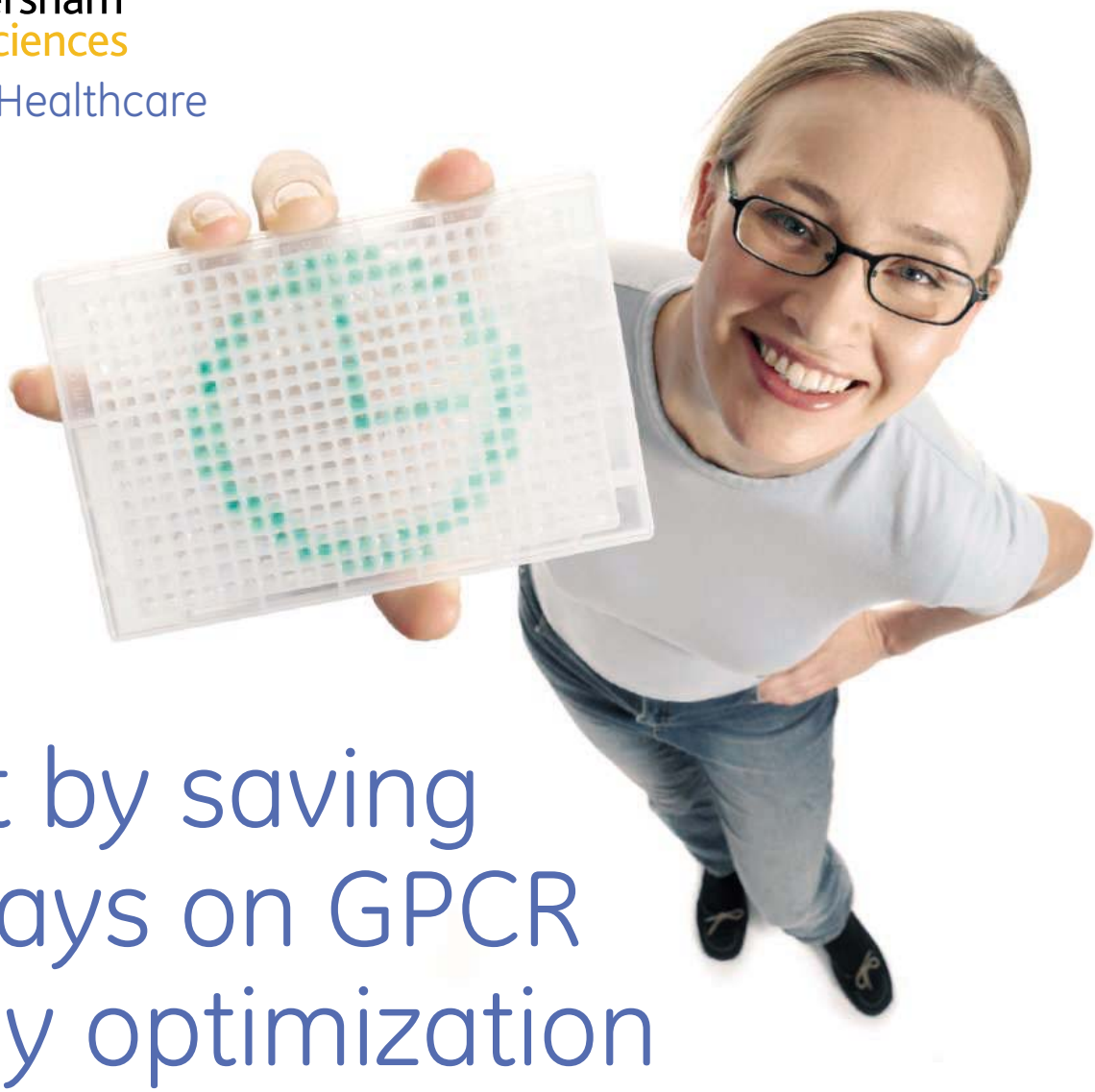

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Science

Special Issue

Women's Health

Volume 308
10 June 2005
Number 5728

Once neglected, studies of women's health are finally coming of age. A special section explores new findings on female-male differences in health and sexuality, as well as conditions specific to women. [Image: Getty Images]

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Reviews

Related Editorial page 1517; Book Review page 1555; Policy Forums pages 1557 and 1558

Special Online Content

www.sciencemag.org/sciext/womenshealth/

science's next wave www.nextwave.org

Career Resources for Young Scientists

Global/MiSciNet: Bouncing Back *E. Francisco*
Three minority women scientists describe the obstacles they faced returning to work after an illness.

GrantsNet: Graduate and Postdoctoral Funding in Women's Health *Next Wave Staff*
Get a sampling of current funding opportunities for research on women's health.

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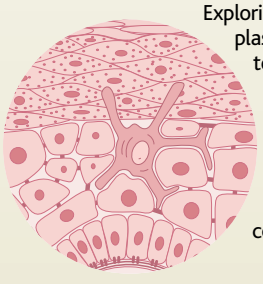
Signal Transduction Knowledge Environment

Editorial Guide: Focus Issue—Women's Health *L. B. Ray, E. M. Adler, N. R. Gough*
This week STKE focuses on signaling involved in cancer and cardiovascular health.

Perspective: Crossroads of Estrogen Receptor and NF-κB Signaling *D. K. Biswas, S. Singh, Q. Shi, A. B. Pardee, J. D. Iglehart*
Signaling by the hormone estrogen interacts with other pathways regulating inflammation and cancer.

Perspective: Rapid, Estrogen Receptor–Mediated Signaling—Why Is the Endothelium So Special? *K. H. Kim and J. R. Bender*



Exploring estrogen signaling at the plasma membrane provides clues to understanding vascular health.

Perspective: Human Papillomaviruses and Cell Signaling *D. J. McCance*
HPV signals through multiple pathways to affect epithelial cell behavior.

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Science of Aging Knowledge Environment

Perspective: The Longevity Gender Gap—Are Telomeres the Explanation? *A. Aviv, J. W. Shay, K. Christensen, W. E. Wright*
Somatic cell selection might promote longevity in women.

Perspective: Why Females Live Longer Than Males—Control of Longevity by Sex Hormones *J. Viña, C. Borrás, J. Gambini, J. Sastre, F. V. Pallardó*
Estrogens might prolong life by increasing expression of antioxidant enzymes.

News Synthesis: Mars and Venus *R. J. Davenport*
Unearthing the reasons that age-related diseases afflict men and women differently might improve health care for both sexes.

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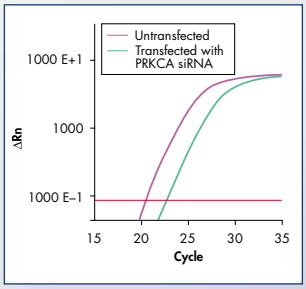

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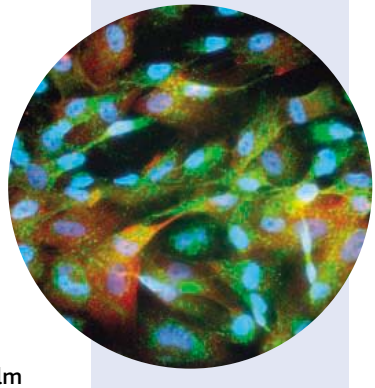

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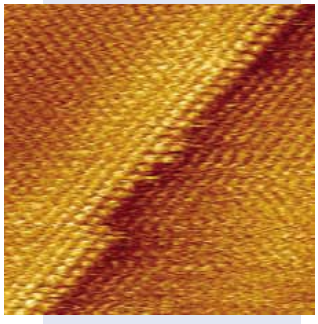

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FIND
FOUNDATION FOR INNOVATIVE NEW DIAGNOSTICS

Almost 2 million people die of tuberculosis (TB) each year, mostly in developing nations lacking access to fast, accurate testing technology.[1] TB is the current focus of the Foundation for Innovative New Diagnostics (FIND), established with funding from the Bill and Melinda Gates Foundation. It is a leading nonprofit organization dedicated to the development of diagnostic tests for infectious diseases in developing countries. For more information, visit www.finddiagnostics.org.

A young girl reveals hope in India, which carries one-third of the global burden of TB.

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Partnering against TB

Twenty-two developing countries carry the burden of 80 percent of the world's cases of TB, the second-leading killer among infectious diseases and primary cause of death among people with HIV/AIDS globally. Spreading through the air when people cough, sneeze, or simply speak, its current rate of infection is one person per second.

BD is pleased to work with FIND to provide equipment, reagents, training, and support to the public health sector in high-burdened countries on terms that will enable them to purchase and implement these on a sustainable basis.

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[1] Source of all statistics cited: *Progress Report on the Global Plan to Stop Tuberculosis*, StopTB/World Health Organization, 2004.

[2] "America's Most Admired Companies" annual survey, 2005; *FORTUNE* magazine, March 7, 2005.

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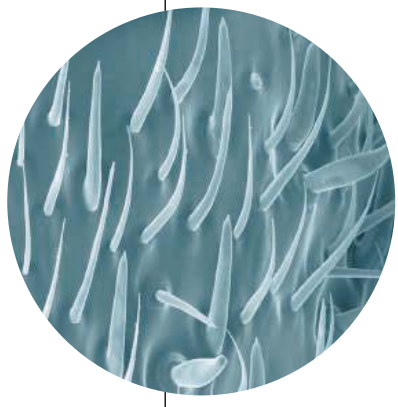

TECHNICAL COMMENT ABSTRACTS

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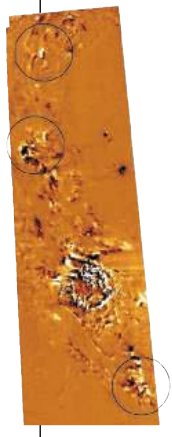

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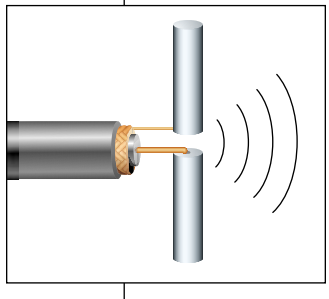

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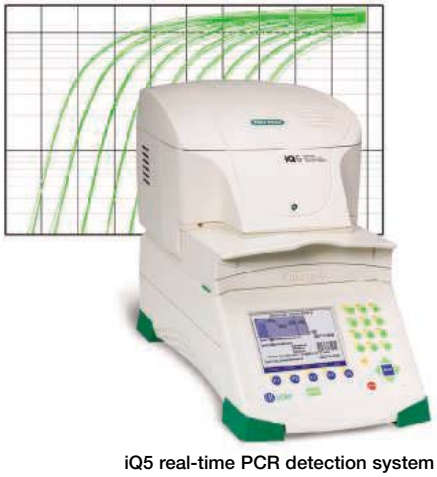

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Gene Therapy Notches Another Victory
Immune system healed in two men, in what may prove to be field's third success.

Chicks Dig Biological Motion
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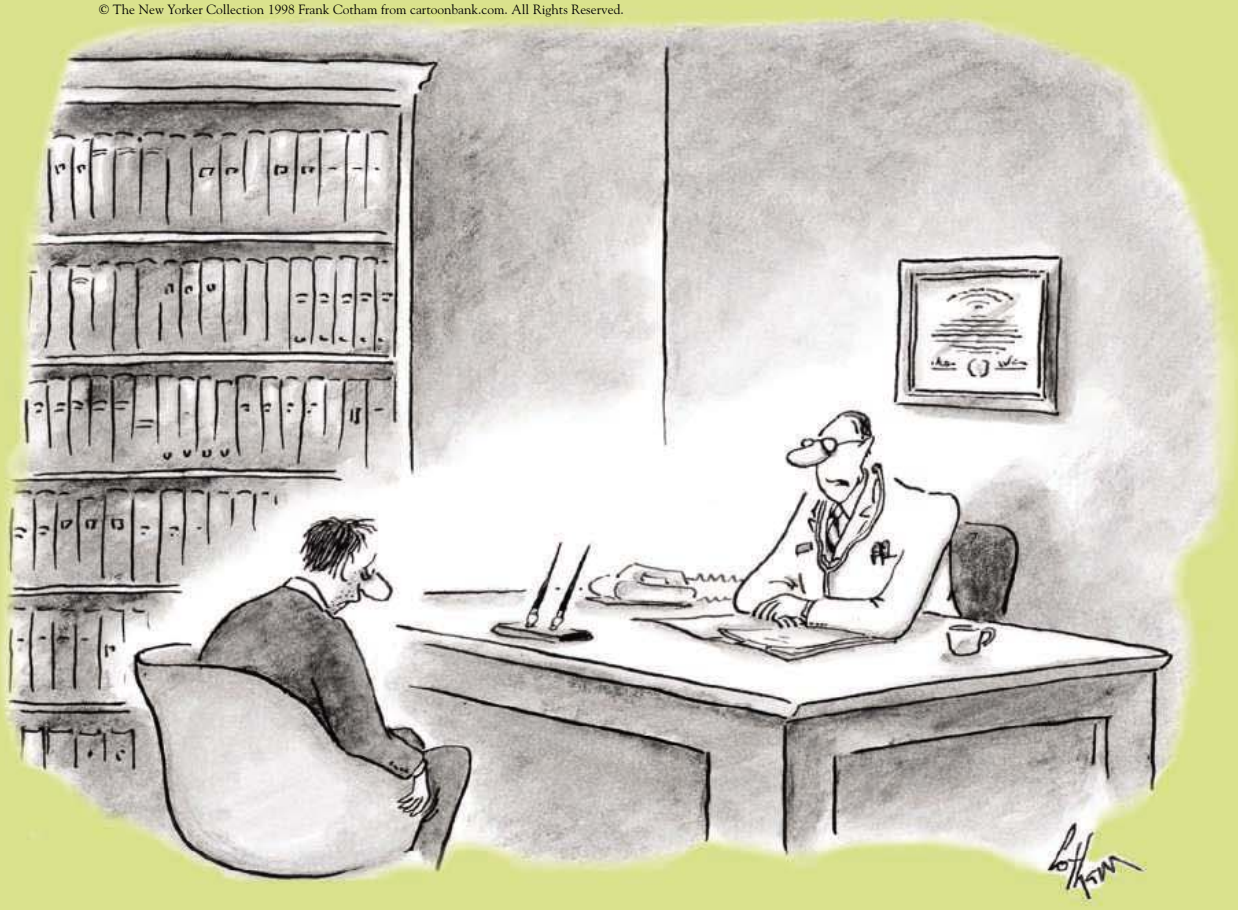
The Supernova That Wasn't
Astronomers expose an "imposter" explosion in a nearby galaxy.

science's next wave www.nextwave.org Career Resources for Young Scientists

Global: Health Issues in the Scientific Workplace—Feature Index *R. Arnette*
Next Wave explores the career decisions scientists must make when dealing with health crises.

Coping with health issues.



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Say NO to Asthma

Physiological nitric oxide (NO) is strongly associated with asthma, although there has been considerable debate about whether it is present in a protective capacity, or contributes to pathogenesis of the disease. Endogenous nitrosothiols (SNO) are NO-carrying molecules present in airway tissue and one, *S*-nitrosoglutathione (GSNO), is depleted in asthmatics. **Que *et al.*** (p. 1618, published online 26 May 2005; see the Perspective by **Gerard**) show that modulation of GSNO levels has direct consequences for susceptibility to an asthma-like condition in mice. Animals lacking an enzyme that breaks down GSNO, GSNO-reductase, showed reduced airway hyperreactivity in response to an experimental allergen. Drugs that reduced GSNO levels reinstated asthma susceptibility in these mice, which suggests that accumulated GSNO was directly responsible for protecting the mice. Thus, NO can help protect against asthma, provided that it is "channeled" through SNOs.

First Impressions

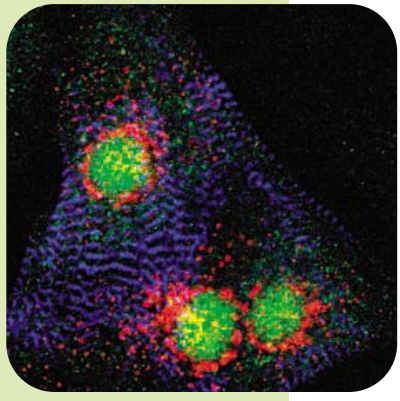
It is sometimes said that first impressions are everything. **Todorov *et al.*** (p. 1623; see the Perspective by **Zebrowitz and Montepare**) provide a remarkable demonstration of how quickly those impressions are formed and what the consequences might be. Several distinct groups of undergraduates were asked to make judgments of relative competence based on 1-second views of black-and-white photographs of unrecognized candidates for the United States Senate and House of Representative contests from 2000, 2002, and 2004. The judgment of competence—unlike those for attractiveness, likeability, or trustworthiness—could be used to predict the outcomes of each of the elections with an accuracy of about 70%.

Super-Sized Food Drops

The amount of food transported to the deep sea floor by sinking particles, as measured with sediment traps, does not seem to be great enough to fulfill the metabolic requirements of benthic organisms that live there. **Robison *et al.*** (p. 1609) conducted a 10-year study in Monterey Bay, off the coast of California, in which a video camera mounted on a remotely operated undersea vehicle was used to measure the vertical distribution and abundance of large organic structures. Discarded mucus feeding structures of giant larvaceans transport approximately half as much carbon to the sea floor as do the small sinking particles that sediment traps capture, and on which past estimate of organic carbon rain rates were based. This finding closes the gap that hitherto has existed between the demand and the supply of food to the benthos for at least this location.

Titin and Muscle Transcriptional Regulation

During muscle differentiation, gene expression leads to the translation of myofibrillar proteins and their assembly into contractile units, the sarcomeres, which are constantly remodeled to adapt to changes in mechanical load. The giant protein titin acts as a molecular blueprint for sarcomere assembly by providing specific attachment sites for sarcomeric proteins, as well as acting as a molecular spring. **Lange *et al.*** (p. 1599, published online 31 March 2005) identify the components of a novel sarcomere-associated pathway that links the sarcomere to the control of muscle gene transcription. The kinase domain of titin initiates a signal transduction cascade that controls sarcomere assembly, protein turnover, and transcriptional control in response to mechanical changes. A mutation in the titin kinase domain affects this signal transduction pathway and leads to a lethal hereditary human myopathy.



Tuning In to Nanooptics

The interconversion of optical excitations between propagating modes and localized light fields first requires the ability to harness the propagating photons. However, designing and fabricating structures on the size scale of the propagating light is challenging. **Mühlschlegel *et al.*** (p. 1607; see the Perspective by **Greffet**) show that antennas can be fabricated from split gold strips and can be designed to be resonant at optical wavelengths. These antennas can focus energy into a small gap region strongly enough to generate a supercontinuum of white light. Optical antennas should find a number of applications in creating spectroscopy and interfaces between propagating and localized optical modes.

From Locust Control to Malaria Control?

There is a pressing need for alternatives to chemical insecticides for targeting adult mosquitoes, the vectors of malaria, owing to the development of resistance and worries about human toxicity (see the news story by **Enserink**). **Blanford *et al.*** (p. 1638) found that treating surfaces with a fungal pathogen of insects reduced the number of mosquitoes able to transmit malaria after an infectious blood meal by more than 100-fold. Fungal infection via contact with netting or solid surfaces was sufficient to cause more than 90% mortality. **Scholte *et al.*** (p. 1641) performed field-based research in rural African village houses, using a fungus in real-life conditions, to target wild mosquito vector populations. Large numbers of mosquitoes could be infected with the fungus, which could inhibit malaria parasite development. Even at moderate coverage rates, a dramatic fall in malaria transmission intensity should be achievable. This biopesticide technology has been adapted from registered technology developed for locust control and could be available for immediate use.

CONTINUED ON PAGE 1515

CREDITS (TOP TO BOTTOM): LANGE *ET AL.*; ROBISON *ET AL.*

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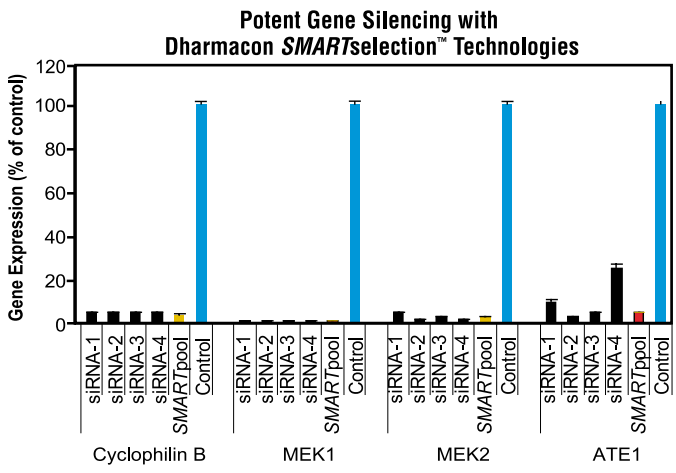

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Apparently Very Fast

Cassiopeia A is the well-studied remnant of a supernova explosion that occurred in 1680. **Krause *et al.*** (p. 1604) used the Spitzer Space Telescope to reveal areas of infrared (IR) emission outside the shell of the remnant that appear to be moving at the speed of light. These apparent relativistic motions may be the result of IR echoes produced by energetic flashes from within the remnant that are heating up the interstellar dust. Such flashes are consistent with emission from objects called soft gamma repeaters or strongly magnetized neutron stars (magnetars).

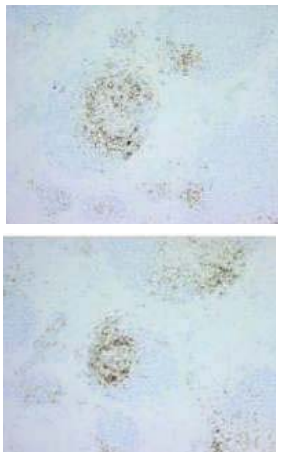
Light Therapy Reduces Stress

The mechanical properties of a polymer depend on both its chemistry, including chain length and distributions, and its processing history. Cross-linking is used to fix a polymer into a particular shape and to stiffen the material by creating chains that are infinitely long. However, this process tends to introduce residual stresses, and there is typically no way to change the shape of a cross-linked network. **Scott *et al.*** (p. 1615) show that ultraviolet (UV) irradiation introduces radicals into the polymer by photocleavaging residual initiator molecules. These radicals then cause the chains to fragment at specific locations along the polymer backbone which can then react to relink the network structure and relieve the residual stress.

North Atlantic Trophic Cascade

Oceanic food webs represent one of the world's most important sources of food for humans. Using data from several different standardized monitoring programs initiated more than 30 years ago, **Frank *et al.*** (p. 1621) establish the existence of a trophic cascade—a series of predatory interactions between different levels of the food chain—in a North Atlantic fishery. The removal of cod by overfishing led to effects that extended across five trophic levels. The large scale of the observed ecosystem change gives rise to pessimism for the recovery of cod in this fishery and perhaps other ecosystems where cod populations have collapsed.

Protozoan's Eleven

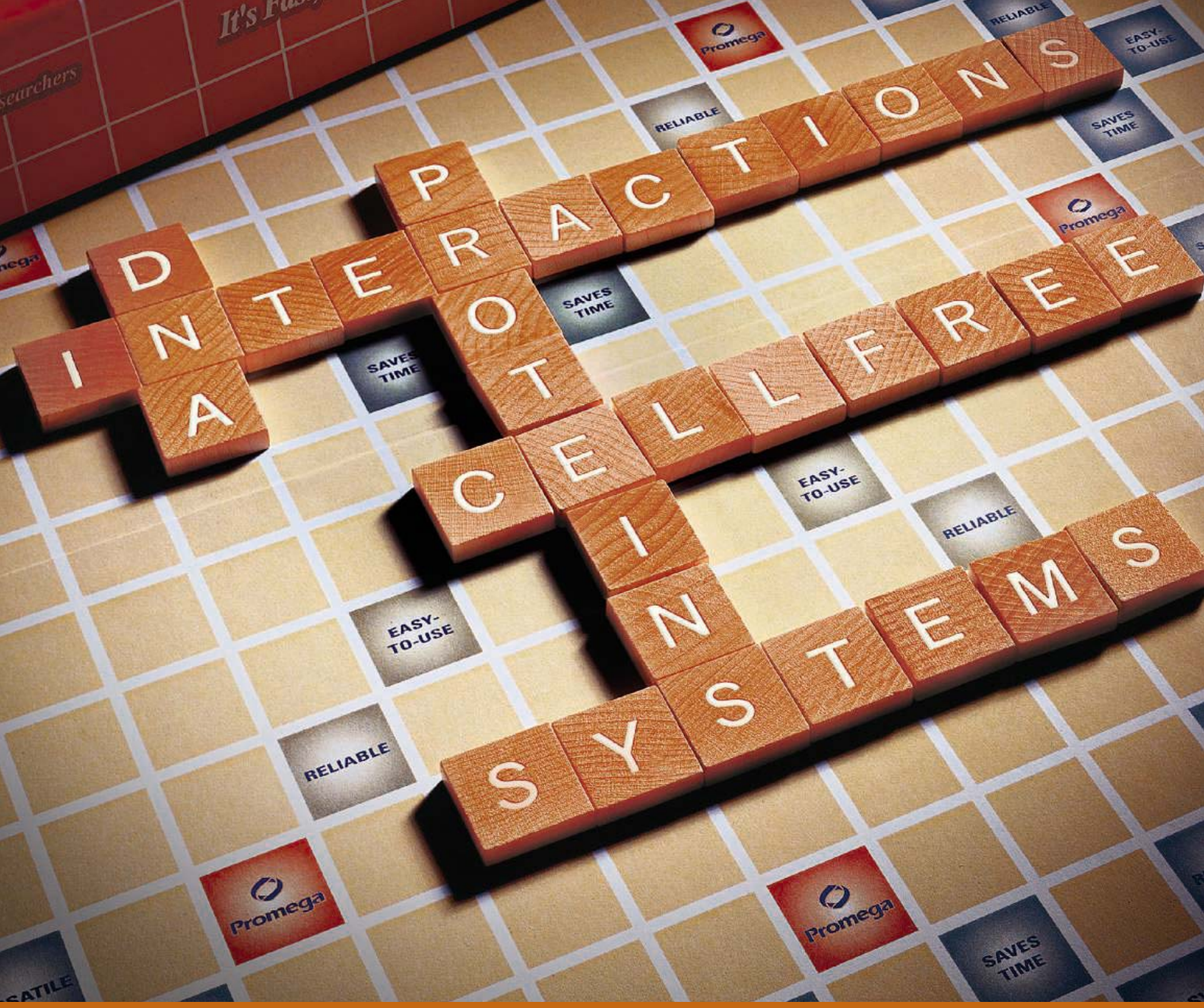


CREDIT: YAROVINSKY *ET AL.*

Mammalian Toll-like receptors (TLRs) are critical modulators of the immune response to pathogens. TLR recognition of bacteria and some viruses are well known, but there have been few examples of recognition of parasite ligands. **Yarovinsky *et al.*** (p. 1626, published online 28 April 2005) describe detection of a profilin-like protein derived from the protozoan parasite *Toxoplasma gondii* by a recently characterized mouse TLR, TLR11. The ligand induced the production of the proinflammatory cytokine interleukin-12 (IL-12) by engaging the TLR signaling pathway. In the absence of TLR11, loss of IL-12 production rendered mice susceptible to *T. gondii* infection. Similar detection of parasite proteins by TLRs may influence the course of immunity against a range of protozoan parasitic diseases.

Ebola Virus: Breaking and Entering

Infection with Ebola virus causes a severe and often fatal hemorrhagic disease, for which there is currently no effective treatment. The molecular mechanisms by which Ebola virus enters host cells and initiates infection are poorly understood. **Chandran *et al.*** (p. 1643, published online 14 April 2005) now show that the endosomal protease cathepsin B is an essential host factor for Ebola virus infection that facilitates viral entry by cleaving a specific protein, glycoprotein GP1, on the surface of the virus. In a cell culture model, inhibitors of cathepsin B activity reduced the production of infectious Ebola virus.



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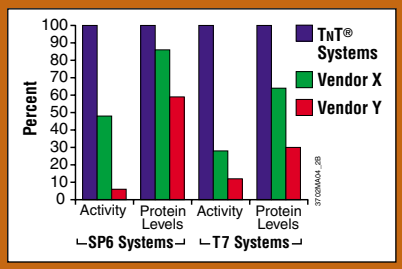

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Wanted: Women in Clinical Trials

Most biomedical and clinical research has been based on the assumption that the male can serve as representative of the species. This has been true in spite of increasing awareness of significant biological and physiological differences between the sexes, beyond the reproductive ones. Women and men differ in their susceptibility to and risk for many medical conditions, and they respond differently to drugs and other interventions. The close of the previous decade saw 8 out of 10 prescription drugs withdrawn from the U.S. market because they caused statistically greater health risks for women than men. Thus, what is true and good for the gander does not seem to be necessarily good for the goose.

After a long history of underrepresentation of women and minorities in clinical trials, federal mandates now require their inclusion in federally funded clinical research in "sufficient [numbers] to provide for a valid analysis of any differences . . . in response to drugs, therapies and treatments." The old paradigm of the "70-kg white male" has finally been replaced by a population sample that attempts to include women and minorities at rates proportional to disease incidence.

This evolution of clinical trials has provided much new information about sex differences in healthy and diseased individuals. Sex is a basic biological variable and should be part of the clinical study design when relevant. Already, a few strategies to standardize methods for conducting such studies in animals and humans have been proposed. However, procedures to enhance the collection and analysis of sex-specific data need to be implemented. For instance, female-specific variables, including the stage of ovarian cycle and use of oral contraceptives or hormone replacement therapy, are factors that may influence intervention outcomes. Moreover, routine pharmacokinetic analysis during early phases of drug development (phases I and II) would be advantageous in determining potential sex differences in dosage recommendations and to prevent adverse responses.

Critics of sex-specific analysis claim that conducting scientifically rigorous trials with enough statistical power to detect sex differences is prohibitive in terms of time as well as cost. Nevertheless, when prescription drugs are withdrawn from the U.S. market because they cause greater health risks for women than men, the cost of not doing such analyses becomes a greater liability for drug companies. It is not necessary that every large, double-blind, randomized, placebo-controlled clinical trial be sufficiently powered to include sex as a covariant, if appropriate preliminary studies are conducted. Small pilot trials can provide information about drug or treatment efficacy for specific populations. These studies can be useful in establishing procedures, protecting against undesirable outcomes, and determining the statistically significant sample size for subgroup analysis. To this end, common protocols are still needed to determine whether subgroup analysis to detect sex differences must be conducted and whether larger clinical trials need to include sex as a covariant.

What is still, unfortunately, lost to the biomedical community is unpublished information that could reveal sex differences. A failure to report such findings leads to the mistaken impression that they do not exist or that they are inconsequential (just last month, the Society for Women's Health Research reported that between 2000 and 2003, the U.S. National Institutes of Health awarded an average of 3% of its grants per year for research on sex differences, while the total percentage of grants awarded during the same period increased by 20%). Cumulatively, this may lead to public mistrust of the drug industry and physicians and ultimately hinder efforts to recruit male and female participants to clinical studies. The need for transparency of such information is being addressed by some resources such as ClinicalTrials.gov, which provides regularly updated information about federally and privately supported clinical research using human volunteers. The creation of an international clinical trials registry could further streamline the application of meta-analytic techniques to help overcome the problem of limited statistical power in small studies.

Researchers foresee a world in which they will be able to read a patient's DNA to gauge the likely course of the person's disease or response to drugs. Until that degree of individualization is possible, patients and doctors must continue to rely on the results of studies carefully designed and analyzed by patient type—including by sex—to obtain the clinical results that are useful and meaningful to the health of both women and men.

Viviana Simon

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10.1126/science.1115616

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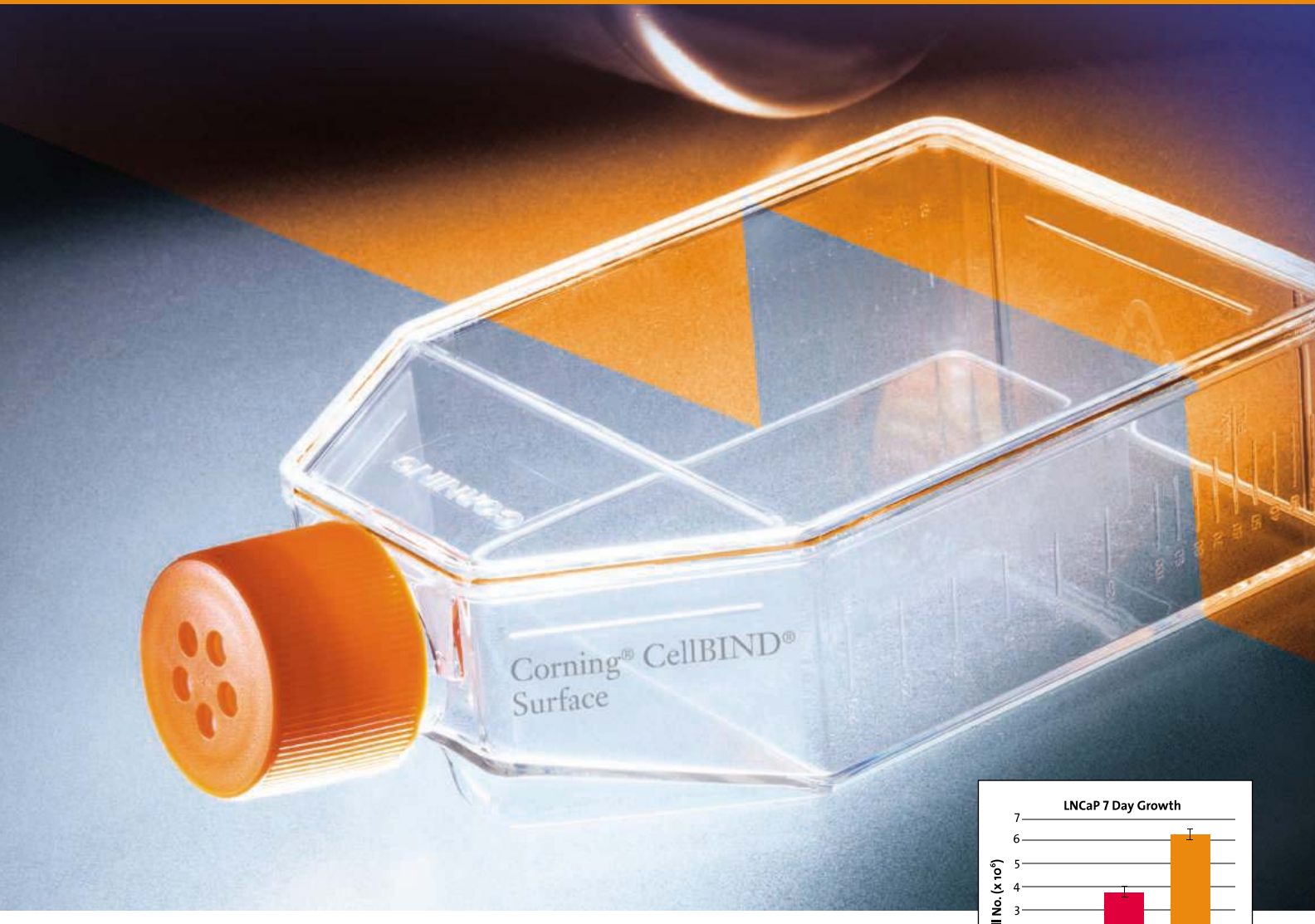

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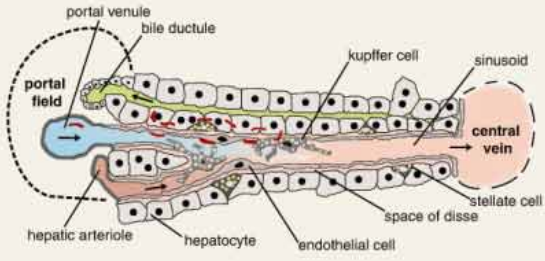
PARASITOLOGY

Infection in Real Time

Malaria parasites (*Plasmodium*) are injected into the mammalian bloodstream by mosquitoes, and the sporozoites travel to the liver, where they elude host immune responses and grow. Frevert *et al.* have taken a technically sophisticated approach to visualizing parasite infiltration of the liver in real time. Mosquitoes, infected with red fluorescent protein–labeled parasites, were continuously fed on a mouse engineered to express green fluorescent protein in cells of the liver sinusoids. Simultaneously, the mouse was held on the stage of a fluorescence microscope, and a lobe of the liver was exposed through the abdominal wall so that the route of the parasites could be monitored. The sporozoites could be seen to glide across the surface of the sinusoidal epithelial cells, to slow down and enter the Kupffer cells, and to use these as a bridge into the liver parenchyma. For up to 15 min, sporozoites traversed destructively through hepatocytes, leaving a trail of necrosis, until finally halting within a hepatocyte and replicating. During these journeys, parasites leave a trail of surface proteins, which tolerize the already immunologically lax Kupffer cells and hence help to shield the invader from host responses. — CA



Route taken by *Plasmodium* (red) from the bloodstream into the liver.

PloS Biol. **3**, e192 (2005).

GEOLOGY

Limits to Weathering

Chemical weathering of silicate minerals removes CO_2 from the atmosphere and therefore provides a key feedback that regulates Earth's climate over long time scales. The rate at which this process proceeds depends on the atmospheric concentration of carbon dioxide, temperature, topography, rainfall, and vegetation; the interaction of all of these factors has made it difficult to establish their separate contributions.

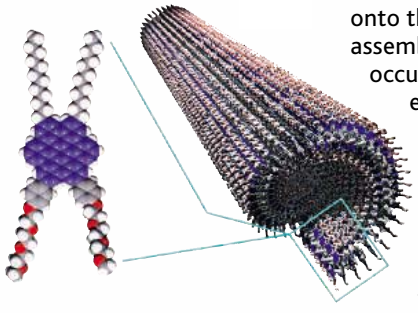
West *et al.* present a compilation of chemical and physical erosion rates in small river catchments and interpret those data with a model for quantitatively discriminating between the controls on silicate weathering by erosion, runoff, and temperature/vegetation in modern environments. They find that silicate weathering is proportional to mineral supply, which limits weathering at lower erosion rates; at higher erosion rates, climatic factors such as temparature and runoff-related kinetics control the rate of weathering. — HJS

Earth Planet. Sci. Lett.
10.1016/j.epsl.2005.03.020 (2005).

CHEMISTRY

Reactive Nanoassembly

One route toward creating nanostructures is first to self-assemble molecules through multiple weak interactions and then to cross-link pendant groups to create stable covalently bonded structures. Jin *et al.* report on a case where the components fail to assemble into larger structures until cross-linking reactions are initiated. Previously this group had shown that large aromatic groups (hexabenzocoronenes) bearing alkyl and triethylene glycol groups could form graphitic nanotubes that were held together by non-covalent interactions. However, when these molecules were derivatized to add reactive propenyl groups onto the glycol chains, no assembly into nanotubes occurred during solvent evaporation. However, when a Ru acyclic diene metathesis catalyst was added to the CH_2Cl_2 solution, ethylene was released and nanosheets formed. — PDS



The hexabenzocoronene (left) and the graphitic nanosheet (right).

J Am. Chem. Soc.
10.1021/ja051859p (2005).

BIOMEDICINE

Giving Stem Cells a Chance

Many important issues surround stem cell and gene therapies, including at what time after birth treatments should be assessed and implemented. This timing is influenced by how early a disease manifests and whether it is considered sufficiently severe to warrant the risk of early intervention.

Escolar *et al.* provide an example of how very early stem cell therapy can enhance the chance of success. The study group consisted of newborns suffering from Krabbe's disease, a rare genetic disorder in which loss of a lysosomal enzyme in cells resident in the central nervous system allows the lipid substrates to accumulate, which results in severe neurological deterioration and death. In an attempt to correct this deficiency, stem cells from banked umbilical cord blood of unrelated donors were transplanted into newborns who either had already started to develop symptoms (142 to 352 days old) or had a family history of the disease but were as yet asymptomatic (12 to 44 days old). In the latter group, survival and neurologic development were significantly improved for almost all graft recipients, with cognitive functions in the normal range. Presymptomatic therapy in this case is likely to have allowed more efficient stem cell replacement of defective resident cells, thus avoiding some of the early toxic effects of the lipid substrates on young neurons and immature neural tracts. — SJS

N. Engl. J. Med. **352**, 20 (2005).

CONTINUED ON PAGE 1521

CREDITS: (TOP) ADOPTED FROM FREVERT *ET AL., PLOS BIOL.* **3**, E192 (2005); (BOTTOM) JIN *ET AL., JAM. CHEM. SOC.* 10.1021/JA051859P (2005)

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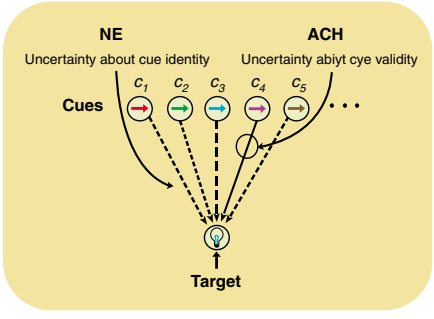
BEHAVIOR

Uncertain Expectations

Life is full of "what-ifs," yet each of us has to collapse multiple uncertainties into a binary yes/no in order to be able to make any decisions at all. Yu and Dayan have constructed a computational model that combines two types of uncertainty—the first incorporates the predictive value of a validated cue, and the second quantifies the likelihood that the existing cue is no longer valid and that a new one needs to be identified—and propose that these are encoded by the neuromodulators acetylcholine (ACh) and norepinephrine (NE); to be precise, by cholinergic and noradrenergic circuits, respectively. In their generalized Posner task, a red arrow points toward the side where the target will appear most of the time, whereas arrows of other colors are randomly oriented. As the predictive value of the red arrow declines, acetylcholine increases. At unspecified times, the red arrow stops carrying information, and another arrow becomes the predictive cue. During this changeover, norepinephrine increases,



A model of neuromodulator encoding of task uncertainties.

signaling the need to search for the cuing color. When the exquisite balance of these systems is disrupted, inappropriate behaviors ensue: A drop in norepinephrine leads to perserverence and a lack of adaptability; conversely, a drop in acetylcholine results in hyperdistractability. — GJC

Neuron **46**, 681 (2005).

CHEMISTRY

No Need for Pores?

It is usually assumed that molecular diffusion through solid materials proceeds by means of pores that are wide enough to allow passage of molecules. Thallapally *et al.* cast doubt on this assumption by showing that water can diffuse through a seemingly nonporous crystal. They determined the structures of calixarene crystals before and after the crystals had been immersed in water for 8 hours. Calixarenes are macrocyclic compounds that can accommodate small molecules; in this present case, in the cleft of a pincer-like configuration. Despite the absence of discernable channels in the crystals, the post-immersion crystals contain one water per host molecule; the lattice structure is otherwise unchanged. The authors rule out crystal dissolution and regrowth because the calixarene is not soluble, even in boiling water, and the same crystal was studied before and after immersion. They conclude that concerted movements of calixarenes might allow the water molecules to diffuse through the crystal until they reach a cavity of suitable size. — JFU

Angew. Chem. Int. Ed. 10.1002/anie.200500749 (2005).

ECOLOGY/EVOLUTION

Not Just a Hanger-On

Among the many characteristic features of tropical forests are large lianas (woody vines) that loop through the canopy and the understory. Unlike trees, they defy easy quantification and so have tended to receive less attention in ecological studies of forest structure and dynamics.

Phillips *et al.* have redressed the balance in a study of liana dynamics in western Amazonian forests, using (i) time series of data on the turnover (defined as death and replacement) of liana and tree stems collected over periods of one to two decades at a number of forest sites, and (ii) a structural inventory of all lianas in an intensively sampled 1-ha plot in southern Peru. The long-term turnover rates of large lianas (with stems >10 cm in diameter) were rapid—about three times those of trees—with annual recruitment and mortality rates exceeding 6%. Infestation with large lianas was associated with higher death rates in canopy trees, though it is difficult to disentangle cause and effect: Liana infestation may hasten death, yet older trees will have been hosts to lianas for longer. Forest primary productivity was also positively associated with liana turnover rates. Although the biomass of lianas is small relative to that of trees, lianas appear to play a disproportionately active role in forest dynamics.— AMS

Ecology **86**, 1250 (2005).

CREDITS: YU AND DAYAN, *NEURON* **46**, 681 (2005)

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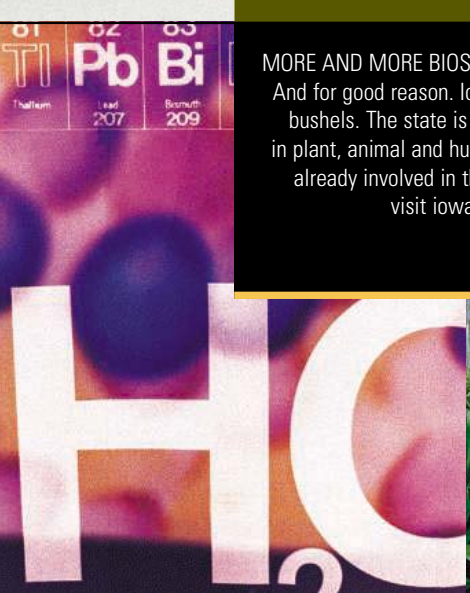

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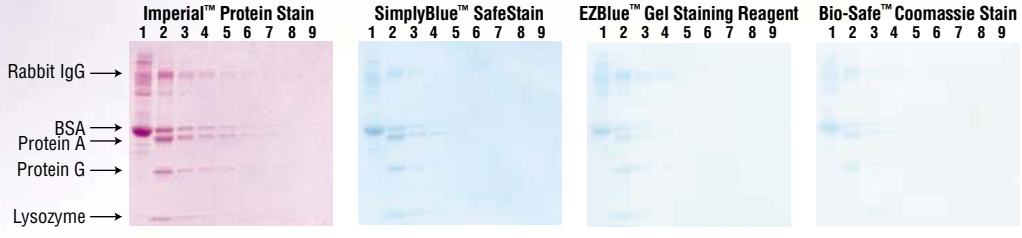

Imperial™ Protein Stain is fast and sensitive. Proteins were separated on Novex 4-20% Tris-glycine gels, stained for 5 minutes and destained 3 x 5 minutes in water. **Lane 1:** BSA only (6 µg), Lanes 2-9 contained the indicated proteins at the following concentrations: **Lane 2:** 1,000 ng, **Lane 3:** 200 ng, **Lane 4:** 100 ng, **Lane 5:** 50 ng, **Lane 6:** 25 ng, **Lane 7:** 12 ng, **Lane 8:** 6 ng and **Lane 9:** 3 ng.

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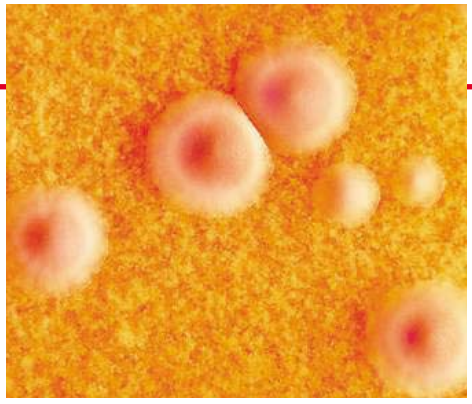
PERBIO

NetWatch

edited by Mitch Leslie



DATABASE

The Microbial A-List

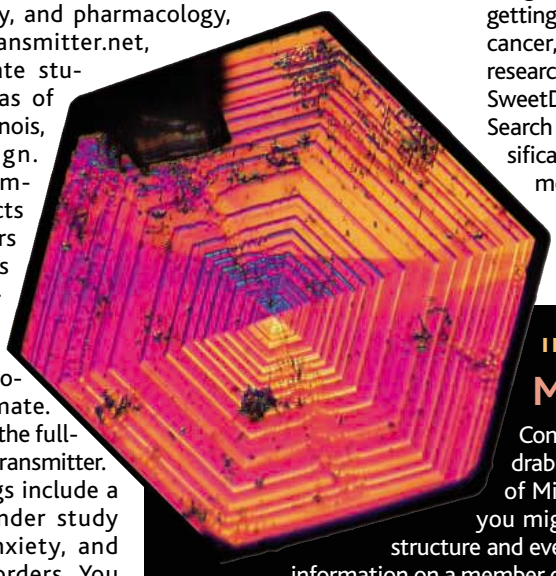
A researcher who wants to name a new species of bacterium is supposed to follow rules set down by an international committee of microbiologists. But plenty of bacterial names floating around the literature haven't met the standards for official recognition. Find out whether a bug's handle satisfies the requirements at the List of Bacterial Names with Standing in Nomenclature, compiled by microbiologist J. P. Euzéby of the École Nationale Vétérinaire in Toulouse, France. As of the last update on 14 May, the site had amassed more than 7000 valid species names, among them *Corynebacterium diphtheriae* (above). The entries include references to the original description, comments on nomenclature difficulties, and other information.

www.bacterio.cict.fr

RESOURCES

Neuroscience in a Nutshell

A well-chosen reading list is a prerequisite for almost any subject, whether it's freshman English or narcolepsy research. To find out what you should read to get up to speed on neuroscience, psychology, and pharmacology, click on Neurotransmitter.net, created by graduate student Shawn Thomas of the University of Illinois, Urbana-Champaign. The site offers compilations of abstracts from recent papers that introduce topics from autism genetics to the connection between migraines and the neurotransmitter glutamate. Some entries link to the full-text articles. Neurotransmitter.net's other offerings include a listing of drugs under study for depression, anxiety, and other mental disorders. You can also browse MetaDB, which links to more than 1000 biological databases on everything from mammalian brain anatomy to the genome of the hepatitis C virus.



neurotransmitter.net

EDUCATION

Physics Through the Centuries

This online exhibit from the Institute of Physics in London lets you zip through more than 5000 years of the discipline's history. Clickable maps summon pop-up windows with brief accounts of major figures and discoveries. You can jump back all the way to the Sumerian culture, which began around 3500 B.C.E. and invented a counting system and basic arithmetic. Or pay a visit to Anaxagoras (circa 490–428 B.C.E.; right), the Greek philosopher who first explained the cause of eclipses and earned imprisonment for arguing that the sun was just a hot rock, not a god. The timeline winds up with modern physicists such as Stephen Hawking and fractal guru Benoit Mandelbrot. "Wormholes" allow you to follow the influence of one thinker on scientists in another time period.

www.physics.org/evolution/evolution.asp

DATABASE

Sugar Storehouse

Long overshadowed by proteins and DNA, carbohydrates are now getting their share of attention because of their roles in immunity, cancer, and other processes. Chemists, glycobiologists, and other researchers can satisfy their craving for basic carbohydrate data at SweetDB from the German Cancer Research Center in Heidelberg. Search the database by categories such as molecular formula, classification, and full or partial structure. You'll nab a 3D image of the molecule, predicted peaks for mass spectroscopy, a list of references, and other results. For another helping of information, sample the site's bibliography of carb publications.

www.glycosciences.de/sweetdb/index.php

IMAGES

Meet a Slippery Customer

Compared with its glittering cousin the diamond, graphite seems drab and grimy. Click on this primer from physicist John Jaszczak of Michigan Technological University in Houghton, though, and you might gain a new appreciation for the substance's intricate structure and even for its beauty. Jaszczak says he created the site to provide information on a member of the carbon family that's often overlooked by mineralogists but that features bonds stronger than a diamond's. Along with backgrounders on graphite's sheetlike structure, the site features a gallery with examples from around the globe, catching the mineral in its many guises: spheres, columns, clumps, and even cones. On this hexagonal sample from California (above), Nomarski differential interference contrast microscopy highlights the spiral pattern that marks the crystal's growth.

www.phy.mtu.edu/~jaszczak/graphite.html

Send site suggestions to netwatch@aaas.org. Archive: www.sciencemag.org/netwatch

CREDITS (TOP TO BOTTOM): CDC; GIRAUDON / ART RESOURCE, NY ; JOHN JASZCZAK

NEWS

This Week

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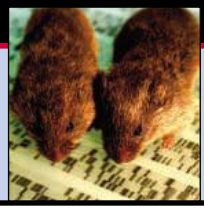
New weapons against malaria

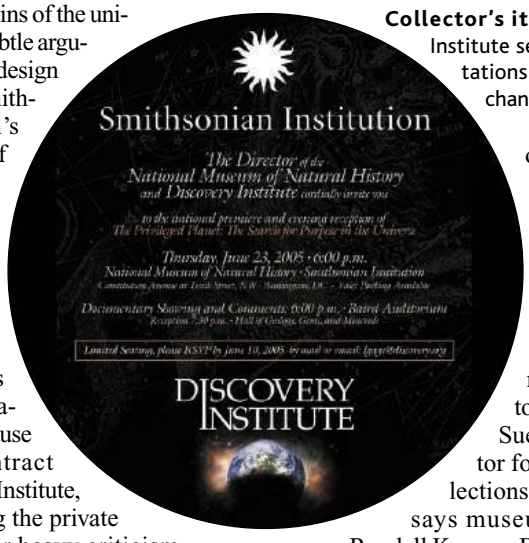


1533
DNA repeats and sexual behavior

SCIENCE AND SOCIETY

Smithsonian Gives Grudging OK To Film Backing ID Argument

A film about the origins of the universe that makes a subtle argument for intelligent design (ID) has left the Smithsonian Institution's National Museum of Natural History with egg on its face. Museum officials say they are reluctantly hosting the upcoming event, even though it violates the museum's scientific and educational missions, because of an ironclad contract with the Discovery Institute, which is sponsoring the private screening. But after heavy criticism from its scientists and outsiders, the museum promises it won't happen again.



Collector's item. The Discovery Institute sent out 1800 coinvitations before the museum changed its stance.

The controversy was triggered by a 26 May story in *The New York Times* that the Washington, D.C., museum would be cohosting a film titled *The Privileged Planet: The Search for Purpose in the Universe*. The film is based on a book by Guillermo Gonzalez, an astronomer at Iowa State University in Ames, and Jay Richards, a philosopher at the Discovery Institute, the Seattle-based nonprofit organization that has been a leader of the ID movement. It presents findings to conclude that the suitability of Earth as a habitat for scientific observation is evidence that the universe was designed for human beings to discover its principles.

In early April, the museum agreed to conduct a private screening of the film in return for $16,000 and co-sponsorship, a requirement for all special events it hosts. But soon after the news broke, museum director Cristián Samper announced that "the content of the film is not consistent with the mission of the Smithsonian Institution's scientific research." Samper said the museum would "honor the commitment made to provide space for the event, but will not participate or accept a donation for it."

The episode has triggered a reexamination of the museum's policies for screening such requests, which preclude events with a religious, political, or commercial message. An initial review by paleontologist Hans Dieter-Sues, associate director for research and collections, came back clean, says museum spokesperson Randall Kremer. But the museum did a second review, Kremer says, "after we realized that people were interpreting our hosting of the event as an endorsement of the Discovery Institute's views."

That review also found that the film fell within the museum's guidelines for such events, says anthropologist Richard Potts. "But it was very clear that the film was trying to situate science within the wider realm of belief," says Potts, who chairs the museum's human origins program. "The idea that human beings have been placed on Earth to discover the principles of the universe is not a position that stems from science; it is a metaphysical and religiously based conclusion."

Having signed a contract, museum officials felt that the event couldn't be canceled. But Potts says the museum may broaden the definition of religious content in its special events guidelines and assign the reviewing to a panel instead of a single person.

Some museum scientists wanted the event canceled. "There's a real concern among many scientists here that the Discovery Institute will use the screening and this association with the Smithsonian to try to gain validity," says paleontologist Scott Wing. But Jack Krebs, vice president of Kansas Citizens for Science, whose members e-mailed protest letters to the museum, says a complete reversal "could have given the Discovery Institute yet another martyrdom story."

–YUDHIJIT BHATTACHARJEE

U.S. SCIENCE BUDGET

VA Asked to Bolster Mental Health Research

The House of Representatives has told the Department of Veterans Affairs (VA) to spend $100 million more on research into the mental health of veterans. But it didn't give the VA any more money, triggering anxiety about what other programs would take a hit.

Stress buildup. Legislators say more research could improve mental health services for returning soldiers.

"It was a no-brainer," says John Scofield, a Republican spokesperson for the spending committee that proposed a reshuffling of the VA's 2006 budget. Scofield was referring to the need to examine the growing incidence of post-traumatic stress disorder, substance abuse, and other serious mental health problems in soldiers returning from Iraq and Afghanistan. The committee chastised the VA for reportedly spending just 7% of its $784 million research budget on mental health and requested a jump to at least 20%. "We're not mandating" the increase, Scofield says, but the legislators warned VA ▶

CREDITS (TOP TO BOTTOM): DISCOVERY INSTITUTE; WALLY SANTANA/AP PHOTO

1534
Stem cells on trial

1541
Physiology at the peak

1543
Physicists puzzle over particles

PSYCHOLOGY

Survey Finds U.S. Mental Health Holds Steady

A nationwide psychological survey that mirrors one conducted in the early 1990s indicates that the mental health of Americans, which suffered a decades-long slide after World War II according to suicide rates and other statistics, hasn't gotten any worse over the past decade or so. Still, some 6% of the population at any given time have mental illnesses that are "seriously debilitating," which makes the U.S. sicker psychologically than other developed nations, according to the survey conducted by the University of Michigan, Harvard University, and the National Institute of Mental Health (NIMH).

Unlike physical ailments, which increase with age, neuropsychiatric disorders generally hit young people, study directors noted at a press conference last week. As a result, said NIMH director Thomas Insel, mental illnesses are greater sources of disability and premature death than are chronic physical disorders.

The new study, called the National Comorbidity Survey Replication, is based on household interviews of 9282 randomly selected adults in 35 states. Its earlier counterpart was the first to assess a nationally representative sample using standardized psychiatric terms.

The new results, published this week in four papers in the *Archives of General Psychiatry*, reveal that over a lifetime, about 46% of the population falls prey to some sort of anxiety, mood, impulse-control, or substance-use disorder. And that's not counting complex psychiatric conditions such as schizophrenia, which are not amenable to a household survey.

Many of the cases documented in the survey are mild, temporary, and never require treatment. However, noted Ronald Kessler of Harvard Medical School's Department of Health Care Policy, even mild cases may become more severe and "accumulate" if not treated early. Hence the high rates of comorbidity: 45% of the subjects diagnosed with one disorder also qualified for another. Depression and alcoholism go hand in hand, for example.

The lag time between onset of a problem and treatment was 6 to 8 years for mood disorders and 9 to 23 years for anxiety disorders. "These new numbers raise the possibility that the stigma against treatment may be even greater than the stigma against the disorders themselves," said Insel.

Nonetheless, the past decade or so of mental health awareness campaigns and the availability of new drugs have paid off to some degree: 18% of those in the study reported getting some treatment in the prior year compared with 13% in the earlier survey. Still, the researchers found that less than one-third of those seeking help had "minimally adequate" care, as defined by guidelines agreed upon by groups such as the American Psychiatric Association.

Lower education levels correspond with poorer mental health, but both blacks and Hispanics, who tended to have less education than the white people surveyed, reported less anxiety and depression. "Minorities in minority communities have particularly low rates," said Kessler, who speculated that they have a "sense of belongingness that many other people don't have."

Despite continued inadequate treatment and long lag times in seeking help, there's a "sea change" occurring in the nation's mental health, added Kessler: "This is the first time we've been able to say there has not been a rise in mental disorders."

–CONSTANCE HOLDEN

Mental Health Survey

MOOD DISORDERS	SUBSTANCE USE DISORDERS
Lifetime prevalence: **20.8%**	Lifetime prevalence: **14.6%**
Median age of onset: **30 years old**	Median age of onset: **20 years old**
Treatment contact: **88.1% to 94.2%**	Treatment contact: **52.7% to 76.9%**
Examples: Depression, bipolar illness	Examples: Alcohol and drug dependence
IMPULSE-CONTROL DISORDERS	**ANXIETY DISORDERS**
Lifetime prevalence: **24.8%**	Lifetime prevalence: **28.8%**
Median age of onset: **11 years old**	Median age of onset: **11 years old**
Treatment contact: **33.9% to 51.8%**	Treatment contact: **27.3% to 95.3%**
Examples: Conduct disorder, intermittent explosive disorder, ADHD	Examples: Phobias, post-traumatic stress disorder, obsessive-compulsive disorder

SOURCE: HARVARD UNIVERSITY

officials that "any significant deviation from this goal shall be reported to the committee … with explanations as to why the goal was not met and remedies being put in place."

The House bill supplies only half of what the VA spends on research, all of it for direct research costs. (The rest, spent largely on salaries and overhead, is dispensed separately.) The legislators approved the level requested by the president, $393 million, which is down from $402 million this year. A VA spokesperson says about 10% of the agency's direct research funds goes to mental health. The Senate has yet to take up the 2006 VA spending bill, which passed the House on 26 May, and any differences must be reconciled before it becomes law.

News of this proposed change swept through the VA community, fueling both apprehension and enthusiasm. "Not to invest this amount of money would frankly be pathetic," says Thomas Horvath, a psychologist and chief of staff at the Michael E. DeBakey VA Medical Center in Houston, Texas, who says he advocated "frequently and loudly" for increased funding while overseeing mental health work in the VA's Washington headquarters from 1994 to 1999.

But other VA researchers, including some in the mental health field, worry about congressional interference in funding priorities and the demand for rapid change. "Mental health is clearly an understudied, underfunded area in the VA," says Alan Bellack, a psychologist at the Baltimore VA Medical Center and the University of Maryland School of Medicine. Still, he wonders whether the community is prepared to absorb such an increase and worries that other disciplines will be squeezed.

The VA currently invests heavily in chronic diseases such as diabetes and cancer, traumatic injuries such as brain injury and amputation, and age-related problems such as dementia and Parkinson's disease, among other areas. The House proposal "will devastate VA research," says a longtime VA scientist who requested anonymity. Legislators didn't specify what to cut.

–JENNIFER COUZIN

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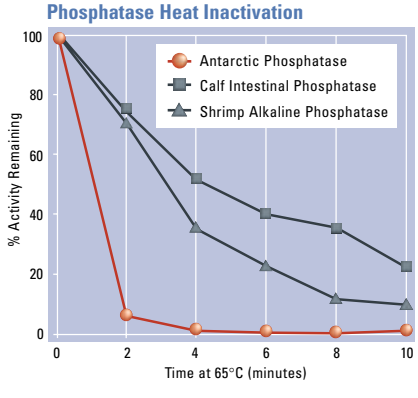

10 units of each phosphatase were incubated under recommended reaction conditions (including DNA) for 30 minutes and then heated at 65°C. Remaining phosphatase activity was measured by p-nitrophenyl-phosphate (pNPP) assay.

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PROTEOMICS

Canadian Global Database May Move To Singapore After Loss of Funding

OTTAWA, CANADA—The world's largest free repository for proteomics data appears headed to Singapore from Toronto, barring an 11th-hour reprieve by Canadian funding authorities.

At stake is the fate of the Biomolecular Interaction Network Database (BIND), which since its inception in 1999 has received $15.5 million from various Canadian agencies. It's an online database containing details of nearly 180,000 molecular interactions submitted by scientists from around the world. Last month its parent group, the Mount Sinai Hospital–based Blueprint Initiative, was forced to lay off half its 68 staffers, and its Canadian bank account will run dry on 30 June. Its future appears to lie in Singapore, which is providing $18.4 million over 5 years starting last summer for a nascent version of the database and has promised more in return for housing the entire database, says principal investigator Christopher Hogue.

CREDIT: THE BLUEPRINT INITIATIVE

Thinning ranks. Canada's Christopher Hogue has had to lay off half the curator staff at a protein database.

Hogue's troubles began after he asked Genome Canada earlier this year for $20.8 million over 4 years to continue running the database. The nonprofit corporation voted thumbs-down, citing what Genome Canada president Martin Godbout says were problems with its "management, budget justification, and financial plan." Hogue says all of those problems—in particular, the requirement that BIND secure matching funding—stem from an unfortunate set of circumstances beyond his control. In particular, he says that a grant of nearly $10 million from an Ontario provincial program has been delayed because of a revamping of the program. Unannounced "rule changes," he adds, precluded him from counting a component of the grant from the Economic Development Board of Singapore to create Blueprint Asia, a Singapore-based component of BIND, ostensibly on the grounds that international contributions aren't de rigueur.

Godbout disagrees with Hogue's analysis. "No project failed the test only because of cofunding," he says.

The upshot, though, is that Hogue has been left scrambling for alternative resources to keep his remaining staff members from joining 33 ex-colleagues on the unemployment lines. And his prospects look no better than those of professional hockey resolving a contract dispute between owners and players and resuming play before next fall. Getting a second shot at funding from Genome Canada would first require the corporation to receive money next spring to hold a new competition. And although the revamped Ontario Research Fund last week issued a call for proposals, the deadline for submissions is not until 14 October. In addition, the fund cannot contribute more than one-third of the overall cost of a project. For BIND, that means other cash-strapped federal agencies would need to chip in to make up the difference.

A move to Asia would compromise the country's nascent biotechnology sector, says Hogue, as well as its reputation as a reliable contributor to international science ventures. If no one steps forward, Canada will lose both trained bioinformatics experts and the scientific prestige that goes with hosting a global project, adds Francis Ouellette, director of the bioinformatics facility at the University of British Columbia in Vancouver. "It's sort of a waste of money to start a project and then basically throw it out and let somebody else reap the benefits," he says. Hogue and others say that BIND's situation also points up the need for greater support for long-lived public data collections (*Science*, 8 April, p. 187).

In the meantime, Hogue is unhappily mulling life in Asia and the fine print of proposed contracts with various Singapore agencies. "I'm a farm boy from Windsor, Ontario. It is my intent to stay in Canada," he says.

But his tone changes when he puts on his BIND administrator's hat. "I can't keep a global database operating without funds," he says. "And I'm not going to shut the database down because, A, it's successful and, B, it's a much-needed resource." **–WAYNE KONDRO**

Wayne Kondro is a freelance writer in Ottawa.

ScienceScope

Ban on Airborne Animals Draws Protest

British Airways (BA) caved in to U.K. activists by agreeing not to transport animals used in medical or scientific research, says Colin Blakemore, chief executive of the U.K.'s Medical Research Council. "I worry that one company folding under pressure would very quickly scare off everyone in the same circle," hurting research, he says. After learning late last year that BA had extended a policy against carrying research animals to mice, Blakemore fired off a letter in February to the airline arguing that its policy would actually hurt animals by forcing them to use less direct routes that require more loading and unloading. The Research Defense Society, which represents U.K. medical researchers, shares Blakemore's concerns and is working to ensure researchers' access to animals.

The airline's change of policy, reported in *The Guardian* in May, has activists claiming success. The airline, for its part, says the move adheres to International Air Transport Association rules and that transporting the animals is not profitable.

–MASON INMAN

House Wants NSF Prizes

U.S. legislators want the National Science Foundation (NSF) to offer innovation prizes for the best research in various fields. The suggestion comes from Representative Frank Wolf (R–VA), the new chair of the spending panel that oversees NSF and NASA. Wolf's subcommittee suggested this week that the National Academies develop "rules and conditions ... with plans [for NSF] to initiate a prize program in fiscal year 2006." No word on the scope of the program, but the panel suggests that NSF sweeten the pot with nonfederal money.

Elsewhere in the House's budget bill for NSF, legislators removed the entire $56 million that NSF had sought for the Rare Symmetry Violating Processes physics project at Brookhaven National Lab in Upton, New York, but approved full funding for its other new facilities. They also granted NSF the flexibility to use non–Coast Guard vessels in Antarctic icebreaking (*Science*, 4 March, p. 1401). Members said they expected NSF to pursue "more economical solutions." NSF is still trying to figure out how the Coast Guard keeps its books, says Karl Erb, head of polar programs, which were tapped for an additional $9 million this year for ship repairs.

–JEFFREY MERVIS

CLIMATE CHANGE

California Sets Goals for Cutting Greenhouse Gases

With the Bush Administration still looking for additional scientific evidence on climate change, states have led the way in proposing ways to reduce further warming. Last week, the high-profile Republican governor of the most populous state in the country weighed in, offering ambitious targets for curbing the state's emissions of greenhouse gases. "I say the debate is over," Arnold Schwarzenegger announced at an annual world environmental festival. "We know the science. We see the threat. And we know the time for action is now."

California exerts a huge impact on the global environment. Its economy is the sixth largest in the world, and the state is the 10th largest emitter of greenhouse gases on the planet. "This is a potentially major political step," says climatologist Stephen Schneider of Stanford University. He and others say the move could spur further action by other states—several in the Northeast are hammering out cap-and-trade systems, for example—and rekindle hopes abroad that the United States might eventually fall in line with the rest of the world on its policies to combat global warming.

Speaking on 2 June in San Francisco at United Nations World Environment Day, Schwarzenegger argued for reductions of greenhouse gases on economic grounds. He cited threats such as the likelihood of reduced water supplies, rising sea level, and more agricultural pests. He also pointed to opportunities for state businesses to develop more environment-friendly technology. "It sends a real signal that … action on climate change is essential to maintaining a strong economy," says Alden Meyer of the Union of Concerned Scientists. California companies might be able to trade emission credits with countries of the European Union, which has begun a cap-and-trade system.

Flexing muscle. California Governor Arnold Schwarzenegger announces an executive order setting targets to cut the state's greenhouse gas emissions.

Although short on details, the executive order lays out three ambitious targets. It calls for lowering emissions to 2000 levels by 2010 and to 1990 levels by 2020. By 2050, the state's emissions would be 80% below the 1990 levels. The short-term targets are not as aggressive as those of the Kyoto treaty but are equivalent to a bill reintroduced last month by U.S. Senators John McCain (R–AZ) and Joseph Lieberman (D–CT). Although Schwarzenegger's executive order didn't mention how to achieve those reductions, he cited 2004 state regulations that require lower emissions from vehicles (which may become stalled in a court battle) and advancing the timetable to 2010 for generating 20% of the state's power from solar, wind, and other renewable sources.

Michael Oppenheimer, an atmospheric scientist at Princeton University in New Jersey, says it would be feasible to achieve the 2010 target (which represents an 11% cut of emissions from today's levels) and the 2020 target (a 25% cut) by quickly adopting such green efforts. The deeper reductions by 2050 may require a cap-and-trade system for greenhouse gases similar to the one implemented by the European Union, he notes. Schwarzenegger has asked the state Environmental Protection Agency to examine options for such a system and report back in January. **–ERIK STOKSTAD**

EUROPEAN UNION

Researchers Lobby to Head Off Threatened Cuts

BERLIN—High hopes among European researchers are turning to worry as political battles threaten to scuttle a planned budget boost and mar the launch of the long-sought European Research Council (ERC).

In April, the European Commission proposed a doubling of the E.U.'s research budget, to €70 billion ($86 billion) between 2007 and 2013 (*Science*, 15 April, p. 342). The plan included €12 billion for a new ERC, which would fund basic research across Europe.

But political tussles over member countries' contributions are threatening to shrink the whole of the commission's proposed €1.03 trillion budget by at least €150 billion. In a proposal put forward on 28 May, Luxembourg's Prime Minister Jean-Claude Juncker, whose country currently holds the E.U. presidency, said the main cuts would come from research programs as well as "structural funds," which build roads and other infrastructure.

"It's very serious," says Helga Nowotny of the Science Center Vienna, who is head of the European Research Advisory Board. Although ERC would still go forward even without the doubling, Nowotny says, a severely reduced budget will diminish its impact. Nowotny and her colleagues sent a letter on 6 June to more than 100 scientific and industrial leaders to lobby their governments to fund the full research proposal. The letter urges recipients to point out "the contradiction between what governments say in favor of research and how they act."

E.U. Commissioner for Research Janez Potočnik says the financial decisions will be a "moment of truth for the E.U." Potočnik was in Berlin on 2 June to try to persuade German leaders—some of the main holdouts in the budget battles—of the importance of research in the E.U. He told *Science* that European politicians say repeatedly that research and innovation should be the highest priority. But protecting subsidies and capping national contributions "turn out to have slightly higher priority."

Some researchers are also concerned about an initial plan for the ERC circulated among the heads of European research councils at a meeting last month in Reykjavik, Iceland, says Ernst-Ludwig Winnacker, head of the German Research Foundation, the DFG. The plan seems to shift power away from a council of independent scientists to the staff of an "executive agency" who answer to the European Commission. Potočnik, however, says the worries are misplaced. All issues of substance, he says "will be decided by the scientific council. The commission will sign off" on the council's decisions.

"It's a matter of trust," Nowotny adds. "Legally it is not possible to give €1 billion to a group of people who have not been elected or even appointed. It must be the commission who takes the ultimate responsibility. But the commissioner has always said he will be the guarantor for the autonomy of the ERC." European scientists will be sure to remind him to keep his word. **–GRETCHEN VOGEL**

CREDIT: JEFF CHIU/AP PHOTO

MICROBIOLOGY

Mosquito-Killing Fungi May Join the Battle Against Malaria

They already kill insects in fields, greenhouses, and gardens around the world. Now, a duo of fungi may also become a new weapon in the fight against malaria. In this issue of *Science*, two research groups report the results of lab experiments and field tests in Tanzania indicating that fungal spores can infect and kill adult *Anopheles* mosquitoes, the vectors of malaria parasites. Applied just like chemical pesticides, sprays containing the spores could be a new, environmentally friendly weapon against malaria, the researchers say.

"They have a pretty strong case," says Christiaan Kooyman, who studies locust control using fungi at the International Institute of Tropical Agriculture in Cotonou, Benin. New control tools are necessary, Kooyman adds, because mosquitoes are increasingly becoming resistant to chemical pesticides.

CREDIT: S. BLANFORD *ET AL.*, *SCIENCE*

Getting fuzzy. Fungi that infect and slowly kill mosquitoes create a fuzzy covering on the parasite-carrying insects.

But whether the fungal strategy is technically or economically feasible remains to be seen, others caution. "I have seen plenty of false technological dawns" in vector control, says Jo Lines of the London School of Hygiene and Tropical Medicine.

That strains of the two fungi—called *Beauveria bassiana* and *Metarhizium anisopliae*—can kill mosquitoes didn't come as a surprise. Both species are used in agricultural biopesticide products, and so many different strains of each fungus exist that there's probably one to kill almost any insect species, Kooyman says. But no one had set such fungi loose on malaria mosquitoes until recently.

In 2003, one group, led by Bart Knols of Wageningen University and Research Centre in the Netherlands and the International Atomic Energy Agency in Vienna, published a lab study showing that spores of several fungi infected *Anopheles gambiae* when applied directly to the insects' bodies. Whereas pesticides kill overnight, these fungi grow slowly, often taking 10 or 12 days to kill.

As they report on page 1641, Knols's team has now tested this idea in the field. They suspended 3-m^2 cloths impregnated with the fungus *M. anisopliae* from the ceilings of five traditional houses in a rural Tanzanian village, collected mosquitoes in the homes for 3 weeks, and kept the insects alive on glucose. Some 23% of female *Anopheles gambiae* mosquitoes became infected, shortening average life span by 4 to 6 days compared to controls from five untreated homes.

The study was much too small to detect an effect on malaria transmission and not designed to do so. But when the team modeled how such results would alter malaria transmission in a village if the cloths were applied year-round, they found that the number of infective bites for the average villager would fall from 262 to 64 annually. In order to make a dent in malaria cases and deaths, that number has to come down much more, to close to one bite per year. But that is feasible by upping the dose and spraying entire walls in many more houses, says Knols's collaborator Kija Ng'habi of the Ifakara Health Research and Development Centre in Tanzania.

On page 1638, a team led by Andrew Read of the University of Edinburgh and Matt Thomas of Imperial College London reports that the true effect of a fungus—in their case, *B. bassiana*—on malaria transmission may be even more pronounced than Knols's data suggest. In lab studies using *Plasmodium chabaudi*, a rodent malaria ▶

ScienceScope

French Science Policy Shakeup

PARIS—Junior research minister François d'Aubert has been ousted as part of the new government formed by Prime Minister Dominique de Villepin following France's overwhelming rejection of the European constitutional treaty. Politician François Goulard, 51, who served as junior transport and sea minister in the last government, now assumes France's top science policy position and will be responsible for higher education, which d'Aubert was not. Bringing the two portfolios together is "good news," says Alain Trautmann, spokesperson for France's researcher protest movement. A long-awaited science reform bill is due to be published next week.

–BARBARA CASASSUS

Final Biodefense Centers Announced

The final pieces of a 10-site national network of biodefense research centers have been put into place. Last week, the National Institute of Allergy and Infectious Diseases announced grants totaling $80 million over 4 years for two new Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases Research.

One center, a consortium led by Colorado State University, will focus on diseases transmitted by animals. Another team, based at the University of California, Irvine, will host clinical trials of vaccines as well as basic research on bioterrorism agents and infectious diseases. Director Alan Barbour says the center will provide "immediate research capability" in case of an outbreak. –JOCELYN KAISER

Stem Cell Institute Faces Possible Vote

California legislators were expected to vote this month on a measure that would tighten conflict-of-interest rules for advisory bodies to the state's new institute for regenerative medicine (CIRM). A committee of overseers at the nascent stem-cell institute moved last week to consider toughening its policies in hopes of heading off the legislation, seen as potentially limiting the participation of experts. If passed by two-thirds of both houses, the proposed constitutional amendment will go before voters in November.

Meanwhile, amid pending lawsuits and financial uncertainty, CIRM this week received $5 million from San Francisco sound pioneer Ray Dolby to help it get started. The institute is also pursuing a $100 million loan.

–CONSTANCE HOLDEN

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parasite, and a mosquito species called *Anopheles stephensi*, the group found that even in surviving mosquitoes, the fungus severely hampered the parasites' ability to develop and mature. "That looks like an important extra benefit," says Wendy Gelernter, a biopesticide consultant at PACE, a company in San Diego, California. In addition, both teams have data suggesting that a fungal infection dampens mosquitoes' appetite for blood meals, making them less likely to pick up parasites in the first place.

Ken Neethling, production director for BCP, a South African company specializing in biopesticides, says his firm may explore the malaria biocontrol strategy commercially; others are interested as well, Thomas says. For now, both teams plan to tinker with the sprays' formulations to see if they can improve infection rates. One key problem: The spores start losing their infectiousness in a matter of weeks. If that can't be solved, the spray would have to be applied over and over. (Pesticides, in contrast, can last a year or longer.) That could be "a near-fatal flaw," says Lines.

Still, these are problems well worth delving into, says Norbert Becker of the German Mosquito Control Association in Waldsee, Germany. As long as malaria kills more than a million people every year, he says, "every new strategy is appreciated."

–MARTIN ENSERINK

GENETICS

In Voles, a Little Extra DNA Makes for Faithful Mates

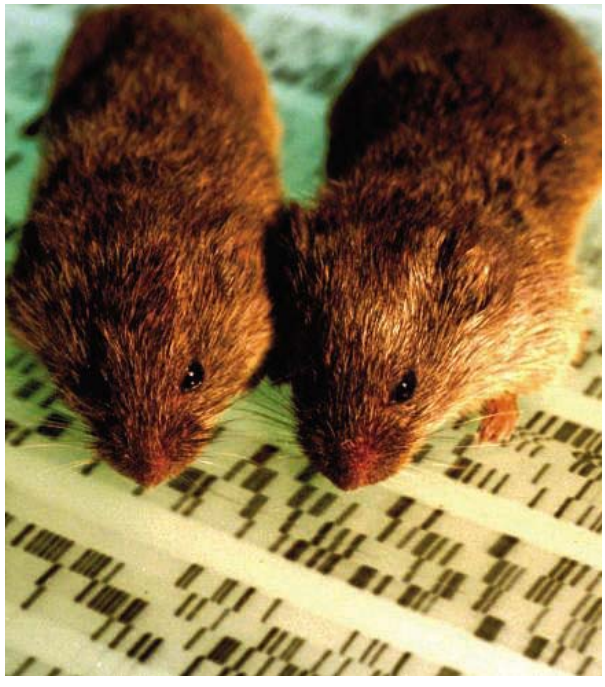
Prairie voles are renowned for being faithful mates, but some individuals are more faithful than others. The difference may lie in their so-called junk DNA.

On page 1630, Elizabeth Hammock and Lawrence Young of Emory University in Atlanta, Georgia, report that fidelity and other social behaviors in male prairie voles seem to depend on the length of a particular genetic sequence in a stretch of DNA between their genes. The longer this repetitive sequence, or microsatellite, the more attentive males were to their female partner and their offspring. Those with shorter microsatellites neglected their mates and pups, at least to some degree.

Although there's no evidence that human infidelity or poor parenting stems from similar variations, Hammock and Young, as well as other researchers, have begun to explore whether microsatellites can account for behavioral differences between people and primates such as chimps and bonobos. The new study's results "will force us to think about these variations in so-called junk DNA and how [they] make for changes in behavior," says Scott Young (who is not related to Lawrence Young), a neuroscientist at the National Institute of Mental Health in Rockville, Maryland.

Microsatellites are genetic stutters, usually just two or four bases long. There can be hundreds of these repeats in a row. They can befuddle the cell's DNA replication machinery, so the number of repeats within one may rise or fall from one generation to the next. And when they are in regulatory regions for genes, their changing lengths may affect the activity of those genes. This can have rapid evolutionary implications, Scott Young points out.

In the mid-1990s, researchers discovered a key microsatellite difference between prairie voles and their more promiscuous cousins, such as the meadow voles. Prairie voles have longer microsatellites near the gene encoding a receptor (V1aR) for the brain chemical vasopressin, and as a result they make more of the receptor than do meadow voles. This was the first clue that these sequences may influence social behavior. Last year, Young's team strengthened the connection when they caused meadow voles to emulate the faithful ways of prairie voles by adding extra copies of the *V1aR* gene to a portion of their brains (*Science*, 7 January, p. 30). "The vasopressin system is likely to be a major player in emotional and cognitive aspects of social bonding," comments Rainer Landgraf, a neuroscientist at the Max Planck Institute of Psychiatry in Munich, Germany.



Honey, I'm home. Sequencing studies revealed that the amount of junk DNA affects how male voles treat their mates.

CREDIT: E. A. D. HAMMOCK *ET AL.*, *SCIENCE*

Now, Young and Hammock, originally one of Young's graduate students and now at Vanderbilt University in Nashville, Tennessee, have found that variations in *V1aR*-associated microsatellites among individual prairie voles influence expression of the gene and overall behavior. They paired and bred voles with long microsatellites and found that the resulting males spend more time licking and grooming their pups than did males with short microsatellites. They also placed males in cages with a female, allowing 18 hours for them to bond, then added a new female. Males with longer microsatellites spent more time with their partners than did those with shorter microsatellites. Taken together, the results "help create a picture of some of the building blocks that allow for the evolution of different levels of social behavior," says Catherine Marler of the University of Wisconsin, Madison.

Evan Balaban, a neuroscientist at McGill University in Montreal, Canada, isn't convinced, however. He argues that, instead of simply showing correlations between microsatellite length and a behavior, the researchers should do transgenic experiments to establish that microsatellites were truly responsible for the different behaviors. Furthermore, "the behavioral effects are small," Balaban adds.

Undeterred, Hammock and Young have already noted connections between *V1aR* microsatellites and primate behavior. Other researchers have associated the length of one of the four microsatellites in the human version of the gene with autism, a disorder of social interactions. In the chimp, this same microsatellite is 360 bases shorter, Hammock and Young note. But in bonobos, which are less aggressive than chimps and form more humanlike social bonds, the microsatellite is nearly identical to the human counterpart.

Even Balaban thinks such intriguing observations deserve follow-up. "Hopefully," he says, "[this will] direct people's attention to studying the role that variation in the control of the regulation of genes plays."

–ELIZABETH PENNISI

At least one company says it is almost ready to try using human embryonic stem cells in patients. But several hurdles remain

Ready or Not? Human ES Cells Head Toward the Clinic

Shortly before Congressman James Langevin cast his vote last month to relax federal rules on funding of stem cell research, the Rhode Island Democrat told his colleagues, "I believe one day I will walk again." Langevin, who has been paralyzed since a gun accident at age 16, pleaded with his colleagues to vote with him. "Stem cell research gives us hope and a reason to believe. … We have a historic opportunity to make a difference for millions of Americans."

With impassioned pleas like this, high-stakes battles in Congress, and billions of private and state dollars pouring into research on human embryonic stem (hES) cells, it often seems their therapeutic applications must be just around the corner. But a careful parsing of the claims from even the strongest advocates reveals the caveat "someday."

How soon that someday might arrive is far from clear. Scientists are nearly unanimous that the study of hES cells will illuminate human development and disease. But whether the cells will actually be used to cure patients like Langevin is less certain. Cell therapies are more complicated than drugs, and hES cells, which have the potential to become any cell type in the body, carry special risks.

"The most sobering thing about [hES] cells is their power," says neuroscientist Clive Svendsen of the University of Wisconsin, Madison, who works with both fetal and embryonic stem cells. The extreme flexibility and capacity for growth characteristic of ES cells makes them ideal for producing large quantities of therapeutic cells to treat, say, diabetes or spinal cord injuries. But these same traits also increase the risk that renegade cells could, as they have in animal studies, cause unwanted side effects, ending up in the wrong place or even sparking cancerous growth. "You have to learn to control that power in the dish" before thinking about putting the cells into patients, says Svendsen.

Pushing ahead. Hans Keirstead hopes his work using human embryonic stem cells to treat spinal cord injuries will enter clinical trials next year.

For that reason, most groups say they are at least five or, more likely, 10 years away from clinical trials. But one company is challenging that timeline. Geron in Menlo Park, California, says its animal studies suggest that stem cell therapy can be safe and might be effective for a select group of patients. The company hopes to start clinical trials of hES cells to treat spinal cord injuries as early as summer 2006. Already, the company is in discussions with the Food and Drug Administration (FDA), which is attempting to set safety standards for the field. Potential treatments with human ES cells face the same difficulties as all cell therapies, notes Malcolm Moos of FDA's division of cellular and gene therapies: There are few standardized techniques to measure the purity or potency of a cell population that would be delivered to a patient.

Most stem cell researchers view Geron's plans with hefty skepticism and caution that a premature rush to patients could seriously damage the already-controversial field. And it is far from clear whether FDA will allow the trial to proceed. But Geron, which funded the researchers who isolated the first hES cells in 1998, has several reasons to push ahead; the company holds a number of patents and exclusive licenses that give it more freedom—and more incentive—to develop possible products from hES cells. And whatever the outcome, scientists agree, Geron's ambitious plans will offer a test case of the hurdles scientists will have to overcome to prove that hES therapies are both safe and effective.

Mending frayed nerves

Even the skeptics say Geron chose a plausible target for the first trial, as spinal cord injuries may be significantly easier to tackle than diseases such as diabetes or Parkinson's (see sidebar, p. 1536). The trials would be based on work led by Hans Keirstead, a neuroscientist at the University

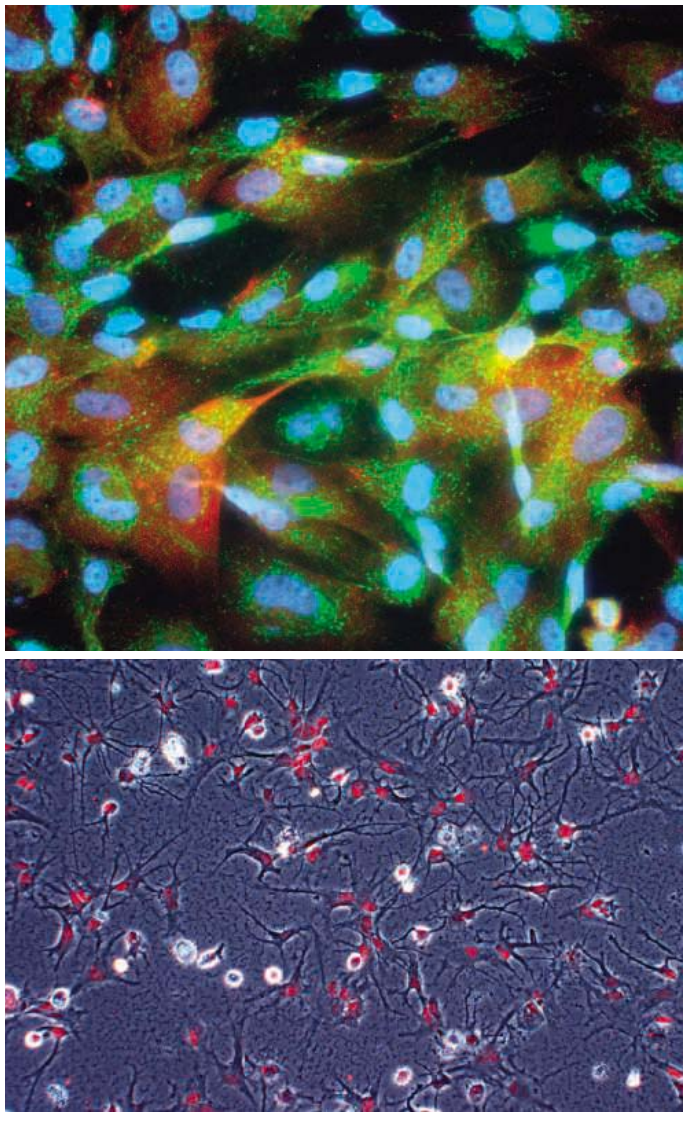
CREDITS (TOP TO BOTTOM): H. KEIRSTEAD/REEVE-IRVINE RESEARCH CENTER; ASSOCIATED PRESS

of California, Irvine, who proved a persuasive spokesperson for the field during the campaign for California's Proposition 71, which provides $3 billion in funding for hES cell research.

During last fall's campaign, Keirstead described his then-unpublished work, showing videos of rats with spinal cord injuries that had regained some mobility after injections of cells derived from hES cells. "I am extremely enthusiastic," Keirstead says. "I am past the point of hope. In my mind the question is when. What we are seeing in these animal models is tremendous."

Keirstead and his colleagues, with funding and technical support from Geron, have developed a protocol that encourages hES cells to differentiate into cells called oligodendrocyte precursors. These cells can form oligodendrocytes, the cells that, among other functions, produce the protective myelin sheath that allows neurons to send signals along their axons. This sheath is often lost during spinal cord injuries.

In a paper last month in the *Journal of Neuroscience*, Keirstead's team reported that these precursors, when injected into the spinal cord, could help improve recovery of rats that had suffered spinal cord injury. The cells aren't replacing injured neurons, Keirstead says, but are encouraging the natural healing process, presumably by restoring some of the myelination. Earlier studies in mice (*Science*, 30 July 1999, p. 754) showed that injecting mouse cells destined to form oligodendrocytes into injured or diseased animals could restore some myelination; Keirstead's team is the first to show that human ES cells can have similar effects.



The right path. Researchers can differentiate hES cells into high-purity neural precursor cells (*top*) that are destined to become the neuron support cells called oligodendrocytes (*bottom*).

CREDITS: H. KEIRSTEAD/REEVE-IRVINE RESEARCH CENTER

For newly injured rats, the results are promising. In animals that received oligodendrocyte precursors 7 days after their injury, the cells survived and apparently helped repair the spinal cord's myelin. Within 2 weeks, treated rats scored significantly better on standardized movement tests than control animals, which had received human fibroblasts or a cell-free injection.

But when the researchers injected cells 10 months after the injury, they saw no effect—sobering news for people like Langevin suffering from old injuries. The cells survived but were apparently unable to repair the long-term damage. For that reason, Keirstead says, Geron's proposed clinical trial would target newly injured patients.

The phase I trial, if it goes forward, will probably include only a handful of patients and, most importantly, Keirstead emphasizes, will not cure anyone. Its primary goal is to show that the treatment can be safe. "The public and scientists must realize that these are the first attempts," Keirstead says. "No one is expecting them to cure. We are expecting them to treat, but we have no idea what the level of response is going to be."

Potential peril

Proving safety is a tall enough order. In numerous animal studies, ES cells from mice and humans have proved difficult to control, differentiating into the wrong kind of cell, for instance, or migrating away from the injection site.

In its spinal cord trial, Geron plans to inject ES-derived cells that can form just a single cell type, an approach that may circumvent some of these problems. For a full recovery, patients are likely to need new neurons as well as other support cells called astrocytes, but using precursors that differentiate into all three types of nerve cells can be problematic. In several rodent studies, partially differentiated mouse ES cells injected into the spinal cord have formed neurons, astrocytes, and oligodendrocytes and have helped animals recover from spinal cord injuries. But more recently, neural stem cells derived from adult animals—which also differentiate into the three cell types—have caused problems. As Christoph Hofstetter of the Karolinska Institute in Stockholm, Sweden, and his colleagues reported in *Nature Neuroscience* in March, neural stem cell treatments led to some recovery in rats' paralyzed hind legs, but the animals also developed a chronic pain sensitivity in their forelegs, which had been unaffected by the injury. In other experiments, preventing the formation of astrocytes seemed to eliminate the side effect, highlighting the importance of proper differentiation, Svendsen says.

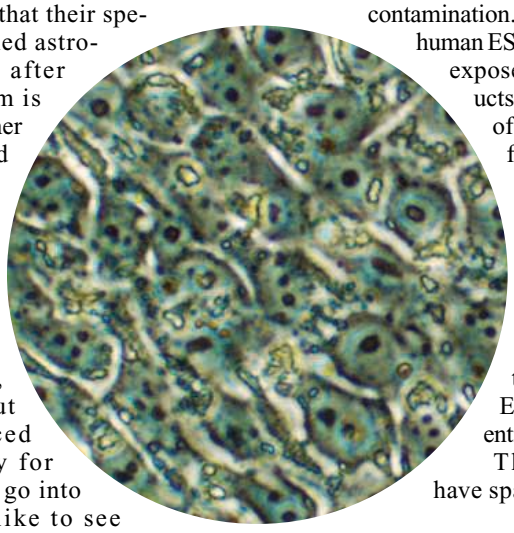
Perhaps the biggest worry is that hES therapies will spur tumor formation. One of the defining characteristics of ES cells is that they form disorganized tumors, called teratomas, when injected in undifferentiated form under the skin of immune-compromised mice. "The ES cell is basically a tumor-forming cell," says neuroscientist Anders Bjorklund of Lund University in Sweden. "This aspect has to be dealt with seriously before the cells are applied in the clinic." Even a benign tumor in the central nervous system would be serious, says Svendsen: "Any sort of growth in the spinal cord is not good news."

But Keirstead believes he has solved those problems. The key, he says, is a differentiation procedure that he claims produces cell populations in which 97% of cells express genes typical of oligodendrocyte precursors. "Teratomas are a real possibility if you put in naïve stem cells," he

acknowledges. "But that is the science of yesteryear. No one is even considering putting in any naïve ES cells." Keirstead and his colleagues say in their paper that they found no evidence that their specialized cells formed astrocytes or neurons after injection. The team is also checking whether any of the injected cells leave the spinal cord. So far, Keirstead says, they seem to stay close to the site of injection.

Keirstead's paper is promising, Svendsen says, but he's not convinced the work is ready for patients. "It didn't go into the detail you'd like to see before a clinical trial," he says. The catch is that it's hard to be sure that a population of several million cells is free of any undifferentiated stragglers. To evaluate the risk of tumors, Keirstead and his colleagues are testing the differentiated cells in nude mice: animals bred to lack an immune system. If the animals live for a year without signs of teratomas, then Keirstead says he will feel confident that the cells are safe to try in humans.

Several teams are making headway addressing another problem: possible animal contamination. To date, almost all human ES cell lines have been exposed to animal products. Cultured cells are often kept alive with fetal calf serum, for instance, and most hES cell lines have been grown on layers of mouse cells called feeder cells, which provide the key proteins that prevent ES cells from differentiating.



Ready for prime time? Geron plans to use one of the original cell lines derived by James Thomson in 1998 in its first clinical trial.

These techniques have sparked worries that hES cell therapies could introduce exotic animal viruses into patients. In response, several teams, including Geron, have recently developed ways to grow new cell lines either on human feeder layers or without feeder cells at all.

But the older cell lines have the advantage of being better characterized, says Geron CEO Thomas Okarma. That's why the company plans to use one of the original lines derived by James Thomson of the University of Wisconsin, Madison, in its first clinical trial. To reduce the risk of contamination, the company has been growing these cells for more than a year without any feeder cells. That may suffice for FDA, which has said that past exposure to animal cells does not disqualify ES cell lines from clinical use as long as certain safety standards are met.

Okarma says Geron can demonstrate that its cells are uncontaminated. His claim is bolstered by a paper by another group published last week in *Stem Cells*. Joseph Itskovitz-Eldor of Technion–Israel Institute of Technology in Haifa and his colleagues tested five hES cell lines and several cultures of mouse feeder cells for signs of murine retroviruses, which lurk in the genome of all mouse cells. Although the team identified receptors for the so-called mouse leukemia viruses, they found no evidence that the virus had infected any of the human cells, even after growing on mouse feeders for years. Animal products still may pose a risk, says Itskovitz-Eldor.

Still Waiting Their Turn

Even enthusiasts agree that Geron's goal—to begin testing a human embryonic stem (hES) cell therapy in patients with spinal cord injury within a year—is a long shot. Prospects are more distant for using stem cells to treat other diseases, such as diabetes, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS). None is likely to reach the clinic for at least 5 or 10 years, most scientists in the field agree. And that's assuming abundant funding and faster-than-expected scientific progress.

Some of the strongest advocates for hES cell research are those hoping to find a cure for type 1 diabetes. The driving force behind California's Proposition 71, Robert Klein, says, for example, that his primary motivation is to find a cure for his diabetic son. Diabetes kills the pancreas's β cells, which regulate the amount of insulin in the blood. Patients have to take frequent insulin injections and face many complications, including kidney failure and blindness. Replacing the missing cells could cure the disease. Initial trials using β-cell transplants from cadavers have shown promise, but side effects and the transplants' limited life span has dampened enthusiasm (*Science*, 1 October 2004, p. 34). And even if the therapy worked perfectly, each transplant requires cells from multiple cadavers. So researchers are looking for renewable sources of cells that could treat the millions of patients who might benefit.

Booster. Robert Klein hopes hES cells will cure his son's diabetes.

In theory, hES cells fit the bill nicely. In practice, however, although several groups have managed to coax mouse ES cells to differentiate into cells that make insulin, no one has yet managed to derive bona fide β cells from either mouse or human ES cells. One reason may be that unlike nerve cells or heart muscle cells, pancreatic cells are some of the last to develop during pregnancy. In mice, the cells appear on day 15 or 16, just a day or two before birth, and in humans, they appear in the 5th or 6th month. "If the road is longer, the possibility of getting lost is much higher," explains Bernat Soria of Miguel Hernández University in Alicante, Spain, who has tried to produce β-like cells from both mouse and human ES cells. Fortunately, says Soria, the cells may not have to be perfect; several types of insulin-producing cells have helped alleviate diabetes symptoms in mice.

But there is no leeway when it comes to safety. Diabetes is a chronic but not inevitably deadly disease, so any cell therapy must be safer and more effective than insulin shots. "We don't have a cure, but we have a treatment," Soria says. "Despite the strong pressure we have from patients and families, the need for cell therapy is not as strong."

Scientists have already attempted to use cell therapies to treat Parkinson's disease, which attacks neurons in the brain that produce the neurotransmitter dopamine, leaving patients increasingly unable to move. In a handful of clinical trials in the last decade, physicians implanted dopamine-producing cells from fetal tissue—with decidedly mixed results. Whereas some patients showed significant improvement, others showed little or none. And some devel-

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Forward thinking. CEO Tom Okarma says Geron, which funded the original derivations of hES cells, will be the first to use the cells in patients.

But the new work shows that "the cells can be tested, and we believe it will be possible to use them clinically."

More recently, researchers identified another potential downside to using mouse feeder cells. In February, Fred Gage and his colleagues at the Salk Institute for Biological Studies in La Jolla, California, reported that hES cells grown with mouse feeders expressed a foreign sugar molecule on their cell surface. Because humans carry antibodies to the molecule, the researchers suggested that it might tag the cells for destruction by the human immune system. If so, then any therapy created with existing cell lines was unlikely to succeed. But Keirstead, Okarma, and others now say that those concerns, widely reported, may have been overstated. Gage and his colleagues noted that the sugar gradually disappears once cells are removed from the feeder layers. Keirstead says that once cells are removed from mouse feeder layers for several months, the sugar disappears. Okarma adds that cells in Geron's feeder-free cultures have no sign of the foreign molecule.

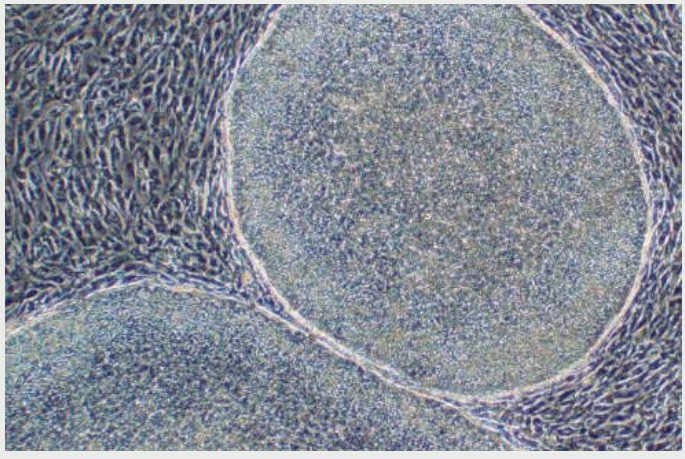
Finally, some scientists worry that ES cells might acquire harmful new mutations in culture, a common phenomenon with almost all cultured cells. Although ES cells "are probably 100 times more stable than adult stem cells in culture, they're not perfect," cautions Mahendra Rao of the National Institute on Aging in Baltimore, Maryland. Such mutations would be particularly hard to detect ahead of time.

Blazing a trail

FDA, meanwhile, is trying to set safety standards for this burgeoning field. The agency announced in 2000 that cell therapies involving stem cells from embryos or adults would be regulated as drugs, not as surgical techniques. That means that researchers will have to meet certain standards of purity and potency. For most drugs, those standards are straightforward to set and easy to measure. Cellular products are much more complicated, oped serious side effects including uncontrollable jerky movements. Scientists aren't yet sure what went wrong, although some suspect that patients may have received either too many or too few fetal cells, which are difficult to characterize in the lab.

Dopamine-producing neurons derived from ES cells could provide an unlimited and well-characterized source of cells. And a trial in monkeys from a team at Kyoto University found that dopamine-producing neurons grown from monkey ES cells could improve animals' symptoms. But before ES-derived cells are tested in Parkinson's patients, scientists need to understand more about how the transplanted cells are behaving in the brain, says neuroscientist Anders Bjorklund of Lund University in Sweden. "The knowledge is just not good enough yet to justify any clinical trials" with hES cells, he says.

Patients and doctors facing the nightmare of ALS may be willing to accept higher risks associated with early hES cell treatments. There is no effective treatment for this invariably fatal disease that kills motor neurons, and patients usually die within 5 years of a diagnosis. But "ALS is an order of magnitude harder than other diseases" to treat with cell therapy, says motor disease specialist Douglas Kerr of Johns Hopkins University in Baltimore, Maryland. Doctors still aren't sure what causes the disease, and even if scientists could coax stem cells to replace the lost motor neurons—"a pretty tall order," Kerr says—any new neurons could be subject to the same deadly assault. More promising, he says, would be a cell or a mixture of cells that might somehow help slow the damage, but no one is sure what that might look like.



Power in a dish. Scientists hope to harness the potential of hES cells to treat a variety of diseases, but years of research remain before that medical potential pays off.

Treating MS has similar challenges, says Hans Keirstead of the University of California, Irvine, who is working with Geron on its possible spinal cord injury trial. "We're much farther away from treating MS with stem cells," he says. Like spinal cord injuries, the disease attacks the myelin sheath around nerve cells, and injected oligodendrocyte precursors have shown positive effects in animal models. But the human situation is more complicated, Keirstead says. Nerves damaged by MS are already surrounded by oligodendrocyte precursors, but something stops the cells from working. Indeed, Keirstead, who is relentlessly optimistic about the prospects of helping spinal cord injury patients, sounds much more sober about the prospects for other patients. "When I look at the work with Parkinson's, MS, and stroke, I think spinal cord injuries are very amenable to these strategies. The rest of the central nervous system is not." **–G.V.**

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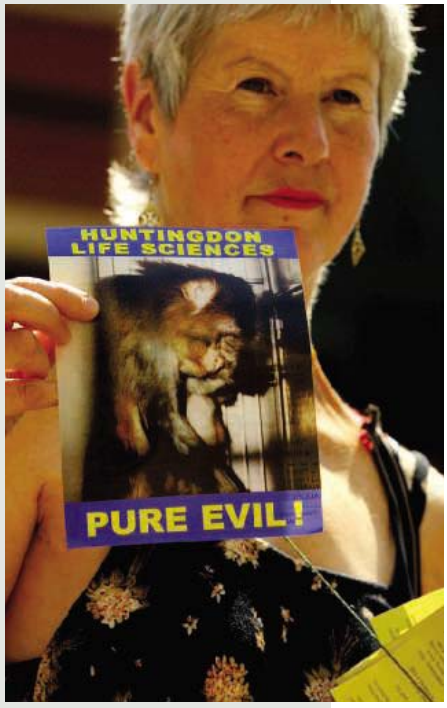
Human Embryonic Stem Cells May Be Toxicology's New Best Friends

Despite their glamorous reputation, human embryonic stem (hES) cells have a long way to go to prove worthy in treating disease (see main text). In fact, the most immediate medical benefit of these controversial cells could be better toxicology studies.

Existing toxicity assays, performed on animals or immortal cell lines generated from animals, often poorly reflect human physiology. For example, chemicals or drugs tested on rodent livers produce the same toxicity in people only about 50% of the time, says Raimund Strehl of Cellartis, a Swedish biotech based in Göteborg. Animal research has also drawn the ire of protesters, particularly in Europe, where some countries, such as Germany, have made the general protection of animals an explicit part of their constitutions.

As a result, pharmaceutical companies are looking for alternatives. Testing candidate drugs on, say, human liver or heart cells might help firms better weed out false leads or identify dangerous compounds before they enter clinical testing. To that end, some toxicologists already use immortal human cell lines or cells taken from cadavers, but both have shortcomings; immortal cells proliferate abnormally, and cadaver cells deteriorate in culture, for example.

Human ES cells might offer a solution, but few published studies have compared these cells, or cells derived from them, to existing assays. "The utility of human ES cells as models for toxicology studies remains speculative, although there is real potential there," says James Battey, who heads the Stem Cell Task Force at the National Institutes of Health in Bethesda, Maryland.



Turning to cells. Animal-rights protests have motivated toxicology research with hES cells.

The search for stem cell–based assays is particularly hot in Europe, fueled by the possibility of E.U. legislation that would require toxicity testing of some 30,000 existing chemicals—and substantially increase animal use. Several companies are exploring the use of undifferentiated and differentiated human or animal ES cells, says Thomas Hartung, head of the European Centre for the Validation of Alternative Methods (ECVAM), part of the European Commission, which is increasing its support of this work. The center has already validated one mouse ES cell assay developed by a German consortium, and Hartung says this and other mouse ES cell protocols could be adapted to hES cells as well.

At least several companies are forging ahead on hES systems. Cellartis, which has established 30 different hES cell lines, is now collaborating with ECVAM scientists to turn such cells into cardiomyocytes—a much-sought-after cell in toxicology research. Geron in Menlo Park, California, has already derived human hepatocytes from its stem cell lines. The company is working with partners to further develop assays and intends to sell the liver cells in 2006 for toxicology studies, says David Greenwood, Geron's chief financial officer. And just last month, James Thomson of the University of Wisconsin, Madison, who originally isolated hES cells, announced that he and two colleagues were starting a company to generate hES cell–derived heart cells for drug testing. If any of these efforts succeed, hES cells may well help make drugs safer for patients—and spare some animals—long before they are used directly in the clinic.

–GUNJAN SINHA

Gunjan Sinha is a freelance writer based in Frankfurt, Germany.

says Moos, and it is less clear what sorts of measures will be used to evaluate cell populations.

Geron is already working, with FDA advice, to gauge the risk of tumors. In ongoing studies, Okarma says, Geron scientists spike the differentiated cells with known amounts of undifferentiated hES cells to determine the threshold level that produces a teratoma in nude mice. He adds that the company has developed "extremely sensitive" assays to detect undifferentiated cells that could ensure cell preparations don't exceed that level.

One of the major questions FDA must decide is whether cell therapies need to be tested in nonhuman primates before they enter humans. Keirstead is not convinced that transplanting cells into primates is a prerequisite for safety. "The question is, are we going to learn any more from putting human cells into monkeys than putting human cells into rats? I'm not sure we know."

FDA's Moos agrees: "Nonhuman primates are not the default choice and in fact are seldom the best choice." For now, Moos says FDA will evaluate this issue, and others, on a case-by-case basis.

Keirstead hopes that these efforts will ease the way for subsequent groups. The first clinical trial will leave behind a set of standards for other teams, he says: "Once you've done that, you've paved the road. You're no longer in 4 × 4s hacking your way through the jungle."

And even if the initial trial doesn't work, Keirstead believes it will advance the field. "An unsuccessful trial is extraordinarily important for moving forward. It's not something that patients and the media want to hear. But it's reality. An unsuccessful trial is scrutinized as much as a successful one." Yet he remains stubbornly optimistic. "We can't eliminate the risk. But I can sleep at night because I do feel the risk is low and the chance of success is high."

Still, many scientists worry that Geron is moving too fast. They point to gene-therapy trials in which one young patient died of an unexpected immune reaction and others developed deadly leukemia. "Gene therapy is a paradigm that we can learn from," says neuroscientist Douglas Kerr of Johns Hopkins University in Baltimore, Maryland. "They have actually induced harm in patients, and that has set back the field."

Others worry that the high-profile political debates may have already set expectations for the field too high, especially among patients who face devastating diseases. Honesty, not hype, is key, says stem cell researcher Bernat Soria of Miguel Hernández University in Alicante, Spain. "My experience is that when you are honest with patients, they are clever enough. The patients tell me, 'We are not sure that there will be a solution for my disease, but please do the research anyway.' People are aware that the road is going to be long."

–GRETCHEN VOGEL

CREDIT: GETTY IMAGES

Culture Systems for Hepatitis C Virus in Sight at Last

The inability to grow this widespread pathogen in laboratories has delayed the development of more-effective drugs and a vaccine

Since the discovery of hepatitis C virus (HCV) in 1988, researchers have made remarkable headway against this liver-destroying pathogen, which infects a staggering 170 million people around the world. Scientists have delineated in fine detail the interaction between HCV, the liver cells it targets, and the immune system. In many people, the drug cocktail of interferon-α and ribavirin eliminates the virus. Studies have also clarified that there are six major genetic families of HCV and that current treatments work better against some so-called genotypes than others. But a fundamental roadblock has stymied scientific progress: HCV has stubbornly refused to grow in laboratory cell cultures—until now.

Green thumb. Frank Chisari's group coaxed HCV to grow with fertilizer from Charles Rice and Takaji Wakita.

Two labs using overlapping but unique approaches this week published evidence online of cell culture systems that can grow relatively high levels of HCV; the virus, in turn, can establish new infections.

HCV researchers are ecstatic. "This is really a great advance," says Michael Gale Jr., who studies HCV at the University of Texas Southwestern Medical Center in Dallas. Gale notes that the new culture systems will finally enable researchers to study critical aspects of the viral life cycle such as cell entry, replication, and packaging into new virus particles, "each of which presents a novel drug target," says Gale. More precise targets, in turn, may yield more effective drugs than interferon-α and ribavirin, which work by unclear, nonspecific mechanisms and fail in a substantial fraction of patients. They are expensive, too, and require a year of injections, so they are of little use in developing countries, where the disease is most prevalent. The culture systems also promise a better way to judge the power of experimental vaccines.

This accomplishment is the result of a friendly but fierce competition between the two groups—one led by Frank Chisari of the Scripps Research Institute in La Jolla, California, and the other by Charles Rice of Rockefeller University in New York City—who shared many critical materials and ended up crossing the finish line neck and neck. As the Chisari team describes in the 6 June *Proceedings of the National Academy of Sciences* (*PNAS*) *Early Edition*, it designed the culture system by using an unusual isolate of HCV and manipulating an immortalized liver cell line. And Rice's group reports online in *Science* this week (www.sciencemag.org/cgi/content/abstract/1114016) similar results achieved by exploiting the same HCV isolate and a closely related cell line.

Six years ago, Ralf Bartenschlager and colleagues at the Johannes-Gutenberg University in Mainz, Germany, laid the foundation for an HCV culture system in a report in *Science*. Bartenschlager's group engineered a small stretch of RNA that codes only for HCV's nonstructural proteins; specifically, the enzymes the virus uses to replicate. This "replicon"—so named because it carries all the information needed to copy itself—replicated to high levels when researchers artificially infected, or transfected, a cell line. But the system had two serious limitations: The replicon replicated efficiently in very few cells in culture, and it could not create a whole, infectious version of HCV.

Bartenschlager (now at the University of Heidelberg) and, separately, Rice recognized that high-level replication might occur only in replicons that mutated and adapted to the cells. Both groups then demonstrated that by introducing mutations into the replicon, they indeed could vastly increase replication efficiency.

Rice and co-workers further increased efficiency by improving the cell line. The replicons apparently only copied themselves in a subpopulation of the cells in culture. So the researchers transfected cells, identified the subpopulation that best supported the replicon, and then used interferon-α to eliminate it. By "curing" the cultured cells, they created a new, optimized cell line.

All the researchers needed next was to engineer a complete viral RNA that used the replicon with the adapted mutations as its backbone. "We hit the wall," says Rice. These engineered HCVs replicated in the improved cell lines and also made proteins, but they did not form a new virus that could establish infections. For unknown reasons, the adaptive mutations in the nonstructural genes reduced the infectiousness of the complete virus.

The race took an odd twist in 2001. Takaji Wakita of the Tokyo Metropolitan Institute for Neuroscience in Japan and his colleagues published a report in the *Journal of Medical Virology* describing an intriguing HCV isolate from an HCV-infected patient who developed fulminant hepatitis and then, oddly, cleared the virus. Wakita's group soon showed that a replicon made from the nonstructural regions of this virus worked in cell lines about as well as replicons that had adaptive mutations. Both the Chisari and Rice labs owe much of their success to clones of Wakita's isolate. "Without his clone, nothing would have happened," says Chisari.

Wakita first teamed up with Bartenschlager, but they had little success. Rice decided to create a chimeric virus that used Wakita's replicon for the nonstructural region

Cured cells. Charles Rice used an HCV drug to create a critical, novel cell line.

CREDITS (TOP TO BOTTOM): LINDA CHISARI; ROCKEFELLER UNIVERSITY

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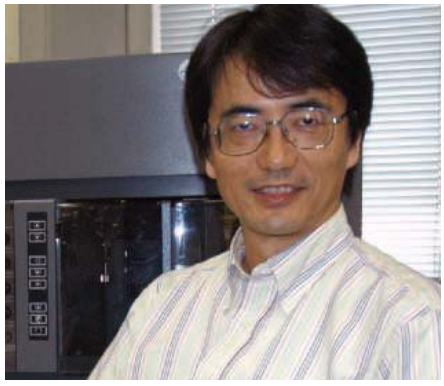
A Match Made in Heaven

and RNA from a different HCV from the same genotype for the rest of the genome. As Rice and co-workers show in their *Science Express* report, this chimeric virus produces high levels of infectious virus when transfected into the optimized cell line they had made earlier.

Chisari collaborated directly with Wakita and used a complete clone of the virus isolated from the patient. "There's something special about that clone," Chisari says. Rice also supplied Chisari with the optimized cell line, which Chisari's group attempted to improve one more time by transfecting and curing it again. In *PNAS Early Edition*, Chisari, Wakita, and coworkers report that when the clone was put into this cell line, it produced roughly equivalent levels of infectious HCV as did Rice's chimera.

What is it that makes Wakita's isolate so special? "That's the main question in our laboratory," says Wakita, who has another paper in press at *Nature Medicine* that describes how he and Bartenschlager ultimately succeeded, too. The secret must reside in the nonstructural region of that isolate, the only common part of the viruses used by each lab, says hepatitis researcher Robert Purcell of the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland. "Eventually that will be sorted out because each gene will be sorted out," says Purcell, who plans to put Wakita's clone into chimpanzees to better understand the relationship between the virus in vitro and in vivo. (Rice also plans to test his chimeric virus in chimps.)



Growth potential. Takaji Wakita isolated an odd HCV that catalyzed the field.

A key limitation of the current advance is that both Wakita's clone and Rice's chimera only contain RNA from one of HCV's six genotypes, which are geographically distributed around the world. "I think this is the long-sought-after culture system, but it's far from as good as we would like it to be," says Chisari. "That's the next frontier," agrees Rice. But for the time being, Chisari, Rice, and other hepatitis researchers at the front of the pack see far more opportunities than limitations.

—JON COHEN

Physiology

Science in the 'Death Zone'

A research team will scale Everest to investigate how a body copes with a lack of oxygen—and possibly learn why some patients do better than others in a respiratory crisis

At 8848 meters, the summit of Mount Everest lies in the "death zone." If a person were whisked straight up to this altitude without supplementary oxygen, he or she would lose consciousness within a minute or so and die soon after. The concentration of oxygen at the peak is only a third of that at sea level, putting it close to the limit for human survival.

It wasn't until 1978 that Reinhold Messner and Peter Habeler achieved the remarkable feat of climbing Everest without bottled oxygen. Others have done it since then, but the ability of climbers to adjust to the lack of oxygen at extreme altitudes still puzzles physiologists—and tantalizes physicians. At a conference in London,* a group of mountaineering medics outlined their plans to investigate the physiological adaptation to hypoxia, or oxygen shortage, in a scientific expedition to Everest. Sister projects at sea level will see if the secret lies in our genes. The Everest team, from the Centre for Aviation, Space, and Extreme Environment Medicine (CASE) at University College London (UCL), says the research could also shed light on why some patients suffering from heart and lung disorders are more vulnerable than others.

The UCL researchers believe their work may elucidate life-threatening conditions faced by some patients in intensive care units, whose blood oxygen levels may fall below 4 kilopascals (kPa). The normal pressure of oxygen in the blood is 12 to 14 kPa, and people usually lose consciousness when it falls below 5 kPa. Climbers summiting Everest without bottled oxygen are estimated to have blood oxygen levels below 4 kPa, says Hugh Montgomery, a UCL cardiovascular geneticist and research leader of the expedition.

As intensive care consultants at UCL, Montgomery and Michael Grocott, who will lead the expedition overall, specialize in treating patients with acute respiratory distress syndrome (ARDS). With ARDS, blood oxygen levels fall dangerously low. Some patients pull through; others do not. Likewise, some climbers and high-altitude dwellers are able to cope with hypoxia; others cannot. Why the differences? Despite much research, says Montgomery, our understanding of the physiological mechanisms underlying the adaptation to hypoxia is "pretty primitive." Attempts to explain human adaptability have focused on changes that enhance the delivery of oxygen to the tissues and cells, such as

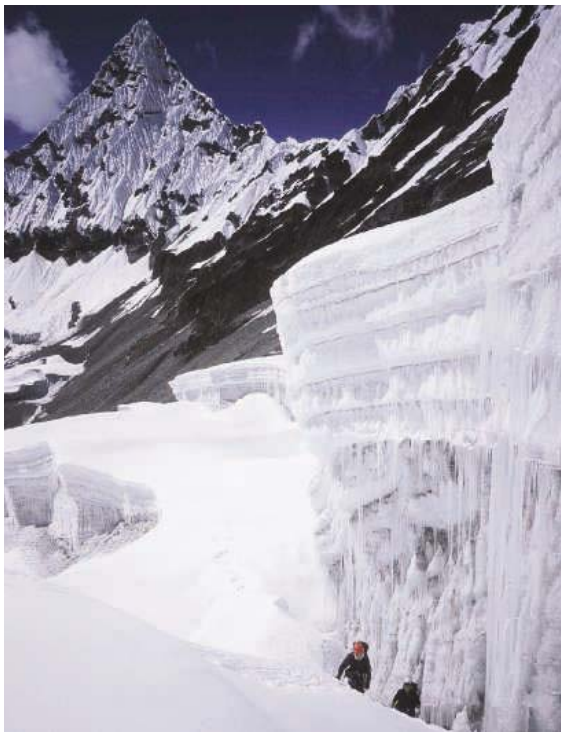
Out of thin air. How climbers survive on scarce oxygen at high altitudes puzzles physiologists.

CREDITS (TOP TO BOTTOM): HIDEKI ITABASHI/METROPOLITAN INSTITUTE FOR NEUROSCIENCE; CENTRE FOR AVIATION, SPACE, AND EXTREME ENVIRONMENT MEDICINE/UCL

* KnO_2wledge: Lessons Learnt from Life at the Limits, University College London, 27 April.

increases in breathing, heart rate, and red blood cell mass. But recent research shows that "something else is going on," says Montgomery—and he contends that it probably has more to do with the biochemistry within cells than changes in oxygen delivery.

Maximum oxygen consumption—the volume of oxygen consumed when a person is exercising as hard as possible—varies a lot among individuals. It is also often less than expected at higher altitudes, says Montgomery. Consider the 2003 study of 12 endurance runners led by physiologist Keisho Katayama of Nagoya University in Japan: The runners' oxygen consumption declined following 3 weeks' acclimatization to a simulated altitude of 4500 m, but they were still capable of doing the same amount of work on exercise tests. This suggests that the body may be able to tweak its metabolism to make cells burn leaner or even reduce oxygen demand by switching some cells off, Montgomery said at the conference.



Extreme heights. Medical researchers from University College London plan to investigate human physiology at its limits while scaling Everest.

If this is true, perhaps ARDS sufferers are experiencing problems not only getting oxygen to their cells but getting their cells to use it more efficiently. It could also mean that it is unhelpful—even harmful—to give them supplementary oxygen. It may interfere with the body's self-protection mechanisms, warns Montgomery. Recent experiments bear this out: When immunophysiologist Michail Sitkovsky of the New England Inflammation and Tissue Protection Institute in Boston and colleagues gave oxygen to lung-damaged mice, it suppressed an anti-inflammatory mechanism normally triggered by hypoxia and caused further damage to the lungs, they report in the June issue of *Public Library of Science Biology*.

Studying adaptations to hypoxia in sick people is problematic; once a patient is in respiratory distress, it's hard to ascertain what's normal. Why not instead see what happens to healthy people when their physiology is pushed to the limit? ask Grocott and Montgomery. They intend doing just that by climbing Everest in spring 2007. Along with remote medicine specialist Sundeep Dhillon, they will lead a team of six to 12 people—all medically trained, experienced climbers—up to the summit. The climbers will conduct physiological and mental performance tests on themselves at several stages along the way. About half the team will attempt the climb without supplementary oxygen. Others will test a new type of closed-circuit breathing apparatus that recycles exhaled air through a filter. The new design could provide a lightweight, compact alternative to conventional open-circuit systems used by patients with chronic respiratory diseases.

This will not be the first time human physiological measurements have been made on Everest. In 1981 physiologist John West of the University of California, San Diego, and his team sampled lung oxygen and carbon dioxide (CO_2) levels at the summit and took a blood sample at 6300 m. West and high-altitude physiologist Michael Ward, formerly of UCL, had earlier conducted the highest exercise tests, at 7440 m on neighboring Mount Makalu, as part of the 1960–61 Himalayan Scientific and Mountaineering (Silver Hut) Expedition, led by mountaineer Edmund Hillary. Still, the new expedition plans to collect unique data, both by working at higher altitudes and by using new technology, Grocott says. Some measurements—including those of blood oxygen and CO_2 levels at the summit—will be groundbreaking.

At several stages on the climb, the team will undertake cardiopulmonary exercise testing, using specially designed recumbent bicycles and devices that measure the amount of oxygen consumed and CO_2 expelled with each breath. This will reveal whether there is an increased efficiency of oxygen use as climbers acclimate, how rapidly it happens, and what makes it happen. They'll test the idea that acclimatized people switch from burning fat to burning glucose, which uses less oxygen for a given energy output.

Montgomery and anesthetist Monty Mythen of the Institute of Child Health at UCL are leading sister projects at CASE that aim to relate performance on cardiopulmonary exercise tests to changes in metabolism and genes suspected of being involved in hypoxia adaptation. For example, Montgomery would like to study high-altitude dwellers and elite climbers to look for variations in the *angiotensin converting enzyme* (*ACE*) gene. *ACE* increases levels of the peptide angiotensin II, which constricts blood vessels, and breaks down the polypeptide kinin, which dilates blood vessels. It also promotes fluid retention by increasing levels of the hormone aldosterone. In 1998, Montgomery and colleagues found that an *ACE* allele associated with lower production of the enzyme was linked to endurance performance in elite mountaineers and army recruits. Subsequent research by Montgomery and others linked the allele to endurance performance in elite athletes and, in 2002, they showed that ARDS sufferers are likely to have the deletion allele, which makes them less likely to survive. Although the physiological effects aren't entirely understood, clinicians may be able to help these patients by chemically mimicking the *ACE*-inhibitory effects of the insertion allele, says Montgomery.

But not everyone is convinced that there is much to be gained from another scientific expedition to Everest or that the results can be applied to hypoxic patients at lower altitudes. "The mountain is a perfect model for the mountain" and not for a medical condition, says physiologist Robert Roach of the Colorado Center for Altitude Medicine and Physiology at Aurora. "There's very little to be learned [about ARDS and other conditions] from going to Mount Everest." Roach argues that it will be hard to separate out the effects of hypoxia from those of other stresses. He and some other experts say it would be better to invest in studies of hypoxia under controlled conditions in low-pressure chambers.

Low-pressure chambers have their drawbacks, counters West. Chambers make poor simulation models because people don't acclimate to low oxygen properly in them, "for reasons we don't quite understand," he says. They're also expensive to run and hold a relatively small number of subjects, adds West. The Everest team expects to secure about $1.8 million for their high-profile trek from private sources. It would be impossible to secure this much for a chamber study, says Montgomery.

Offering to be your own guinea pig, while climbing Everest and experiencing hypoxia, is not for the faint-hearted. But as West says: "Science is full of educated guesses. There's nothing like making the measurement."

—FIONA PROFFITT

Fiona Proffitt is a freelance science writer in Oxford, U.K.

CREDIT: CENTRE FOR AVIATION, SPACE, AND EXTREME ENVIRONMENT MEDICINE/UCL

KEK Researchers Catch Glimpse Of Outlandish Particles

Enigmatic results are forcing physicists to consider that their collision data may bear the footprints of an unexpected newcomer and a long-sought hybrid

"When you have eliminated the impossible," said Sherlock Holmes in *The Sign of Four*, "whatever remains, however improbable, must be the truth." High-energy physicists are now coming to improbable conclusions about two recently discovered particles known as the X(3872) and the Y(3940).

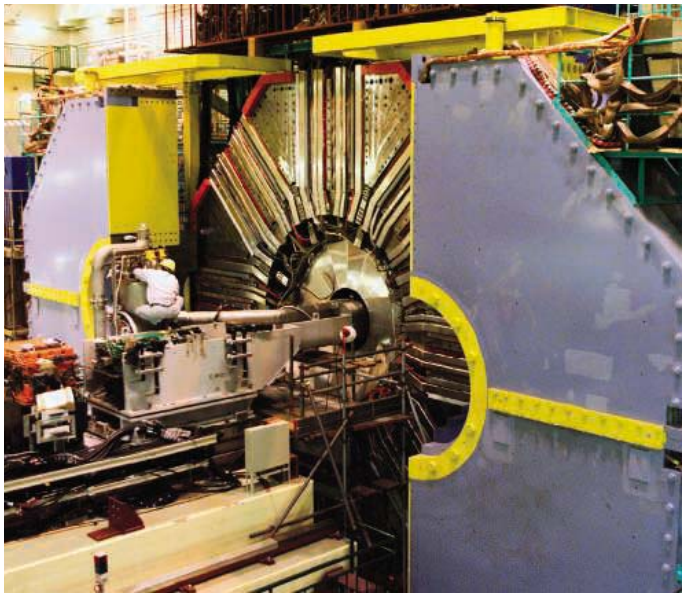
"If you take the results at face value, it looks like the X(3872) is something new," says Stephen Godfrey, a physicist at Carleton University in Ottawa, Canada. Scientists have been unable to reconcile the properties of the particle and the Y(3940) with simple predictions from standard theories about how quarks and gluons make composite objects. They suspect that the X(3872) may be the first member of a new class of particles not predicted by theory, and the Y(3940) might be quarry physicists have hunted for years: the hybrid meson.

Scientists working on the Belle experiment at the KEK laboratory in Tsukuba, Japan, caught the first glimpse of the X(3872) in 2003. They were using an accelerator to smash together electrons and positrons, producing vast numbers of B mesons—moderately heavy particles—which then decay into lighter particles. By studying the products of such decays, scientists at Belle and at the rival BaBar experiment at the Stanford Linear Accelerator Center are figuring out fundamental properties of the building blocks of matter (*Science*, 22 August 2003, p. 1026). But in the KEK data, there was a curious spike.

In a certain type of data plot, particles created in the decay of B mesons show up as a peak in the graph. When studying B mesons that had decayed into a combination of particles known as J/ψs and pions, the Belle researchers noticed that there appeared to be a peak at 3872 MeV—evidence of a new species roughly four times as massive as the proton. This was a problem.

"If it was a standard particle, theorists told us that there should be a strong decay" into a gamma ray and another type of particle, says Stephen Olsen, a physicist at the University of Hawaii, Manoa, and member of the Belle collaboration. But the new particle didn't seem to decay in that manner.

Baffled, the experimenters took a closer look. Theorists had predicted about a dozen as-yet-unidentified but otherwise ordinary particles around 3872 MeV, so they had to eliminate those candidates before they could conclude that the mystery particle was something entirely new. "There's a lot of slop in the models," says Godfrey. "[The X(3872)] was not quite what was expected, but one should be a bit conservative and ask what are the conventional states that it could be."



Monster maker. Particle collisions at Belle created some oddities.

CREDIT: COURTESY OF KEK

By studying the ways that the new particle decays and other characteristics, such as the angles at which the decay products fly apart, the scientists crossed all the standard particles off the list. "They seem definitely to have seen something inconsistent" with conventional particles, says Estia Eichten, a physicist at Fermi National Accelerator Laboratory (Fermilab) in Batavia, Illinois.

So what is this new particle? The most likely possibility, Olsen says, is that two mesons known as D^0 and D^{0*}, each made up of a charm quark and an up antiquark, cleave together and make a more complex object—much as a proton and a neutron can stick together to form a nucleus of heavy hydrogen. Eichten agrees that the sticky-meson hypothesis explains the properties of the X(3872) quite well.

The Y(3940), a slightly heavier mystery particle found by the Belle team, also fails to decay as theory says it should. "It's supposed to [almost] always decay to a D^0 and D^{0*}," says Olsen. Instead, the particle gives rise to a J/ψ and other products. This strange decay pattern, like that of the X(3872), is leaving physicists scratching their heads. "It may be a completely new kind of particle," says Olsen. Although conventional particles haven't yet been ruled out, he adds, this time physicists "don't have such a big list to track down" before concluding that the Y(3940) is something completely new.

If so, its newness is quite different from that of the X(3872). Unlike the X(3872), whose graph shows a nice clean signal with a sharp and narrow peak, the Y(3940) has a broad peak that's less distinct. A two-meson amalgam doesn't explain that signature and other properties. But a long-predicted but never-seen creature does: the hybrid meson.

A meson can be thought of as two quarks attached by a rubber band; the band is the strong-force "glue" that holds the meson together. "But say you pluck that elastic band so that it's vibrating," says Godfrey. "It's a higher, excited state of the meson. You can calculate the predicted mass of these states." This is a hybrid meson, and its excited glue would make it behave like a cross between ordinary quarky matter and matter that's made up of glue. A hybrid meson would have the unusual decay properties observed in the Y(3940), but it's too early to rule out more mundane explanations.

Physicists around the world are trying to follow up on these discoveries. Experiments at Fermilab have also spotted the X(3872) and are looking for the Y(3940). Belle physicists are working hard to rule out conventional explanations and bolster the case for the unconventional ones—and to chase down a handful of other strange particles that have been cropping up. "There's a few others we're looking at that we haven't reported yet," says Olsen. Although it's still too early to declare that physicists have ventured into uncharted territory, the game is definitely afoot.

–CHARLES SEIFE

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Edited by Constance Holden

Cetacean Culture?

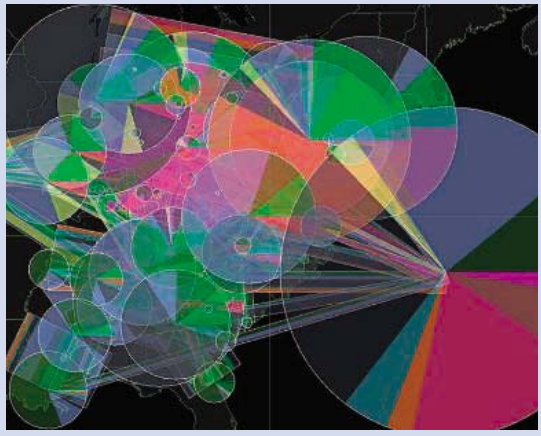
Researchers have long known that dolphins are smart. But a new study showing that dolphins use sponges as tools suggests that they have culture as well. If correct, it would be the first unambiguous demonstration that a marine mammal can transmit information from one generation to the next.

For the past 20 years, marine scientists have been monitoring a population of more than 850 bottlenose dolphins in Shark Bay, Western Australia. A small subgroup has been observed breaking sponges off the bottom and wearing them over their snouts, apparently to probe into the sea floor for fish. A team led by evolutionary geneticist Michael Krützen of the University of Zurich, Switzerland, and including researchers from Australia, Canada, and the United States, obtained genetic data from tissue samples taken from 185 of the dolphins, 13 of whom were spongers. The team reports in this week's *Proceedings of the National Academy of Sciences* that the 13 spongers, all but one of them female, are closely related to one another and probably share a recent ancestor.

Baby dolphin with nose sponge.

The team considered two alternative explanations: Either sponging was a genetically determined behavioral trait, or mothers were passing on the sponging tradition to their daughters. But an exhaustive analysis of genetic data from the 13 spongers and 172 other dolphins, which considered 10 possible ways the behavior could be inherited, including through sex chromosomes and mitochondrial DNA, came up empty-handed. That left cultural transmission as the only viable hypothesis, Krützen's team says.

Psychologist Andrew Whiten of the University of St. Andrews in Fife, U.K., who has studied culture in chimps and orangutans, calls the research "an exciting addition to the catalog of what we can be increasingly confident are culturally transmitted forms of tool use in nonhuman populations."



Go With the Flow

Who says operations research and financial engineering can't be beautiful? Princeton students Warren B. Powell and Belgacem Bouzaiene-Ayari put together this graphic, one of the winners of Princeton's first annual Art of Science Competition, from a "dynamic asset allocation" problem in freight transportation. It's part of a "stochastic, dynamic programming model ... for stochastic, integer multicommodity flow problems," they explain. (See more winners at www.princeton.edu/artofscience/gallery.)

Heavenly Art

Art in space got its launch in 1969, when Apollo 12 carried a postage stamp–sized tile of tiny drawings, including an Andy Warhol rendering of a penis. "We are regularly solicited by artists to send pieces into space, or for astronauts to perform something," says Dieter Isakeit of the European Space Agency (ESA) in the Netherlands, who oversees European users of the international space station. But ESA's approach to choosing projects has been haphazard, he admits.

Now ESA has signed a $90,000 contract with Arts Catalyst, a London-based organization, to consult with artists on how they might make use of the space station and astronaut training facilities. ESA favors projects that "change the states of mind of more people, or make more people happy," Isakeit says. Sending artists themselves into space costs too much, he says, but one idea floating around would involve exposing astronauts to "different sounds that induce the idea of infinity."

Artist-performer Ricky Seabra, who has proposed a module for art and performances on the space station, says "space is not a realm for science alone." And the artist's realm is not art alone either: Seabra plans to apply for astronaut training.

Cosmonaut Alexander Polischuk interacts with a sculpture in the Mir Space Station in 1993.

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Edited by Yudhijit Bhattacharjee

JOBS

Fresh start. A longtime academic with an interest in health policy is the new head of research at the Department of Veterans Affairs (VA) in Washington, D.C.

Cardiologist Joel Kupersmith, 65, succeeds Nelda Wray, who left in late 2003 amid charges of corruption and intimidation. The VA inspector general later confirmed that Wray had mismanaged nearly $1.7 million in VA funds (*Science*, 2 April 2004, p. 29).

Since Wray's departure, VA research has "gone from mismanaged to rudderless," says virologist Douglas Richman of the VA San Diego Healthcare System and the University of California, San Diego. "What we really need is someone who is both administratively competent and a proponent of research."

Kupersmith, a former medical school dean at Texas Tech University in Lubbock and most recently a scholar in residence at the Association of American Medical Colleges and the Institute of Medicine, has pledged to evaluate grants fairly and fund the "best possible research."

Got any tips for this page? E-mail people@aaas.org

PIONEERS

A perfect fit. Wayne Daniel's fascination with building geometrically symmetrical objects began 40 years ago while making a tetrahedral kite for his son. Since then, the former General Motors physicist has spent countless hours crafting three-dimensional puzzles.

The 78-year-old Daniel's latest creation is a set of the five platonic solids—the tetrahedron, cube, octahedron, dodecahedron, and icosahedron—nested one inside the other. Daniel modeled the puzzle on a computer, using classical algebra and geometry to optimize the dimensions of each solid, and then fashioned its 41 interlocking wooden pieces with a table saw and glue. "It was both a theoretical and a woodworking challenge," says Daniel, who lives in a house that he designed in Genoa, Nevada.

Daniel has patented some of his puzzles, which are used in schools to teach algebra, trigonometry, and geometry. His next challenge is designing a puzzle based on the surface of a soap bubble, a shape he finds aesthetically pleasing.

Oncology umbrella. Oncologist David Khayat has been named director of France's new National Cancer Institute. The $125 million institute, inaugurated last month in Paris, will try to stitch together what Khayat calls "very, very divided" efforts in cancer research, prevention, and care. It's one of 70 initiatives in a 5-year cancer plan announced 2 years ago by French president Jacques Chirac.

Khayat comes from the Pitié-Salpêtrière Hospital in Paris and was a driving force behind the 2000 World Summit Against Cancer. "He is an excellent choice," says David Kerr, director of the National Translational Cancer Research Network in the United Kingdom. "He enjoys widespread support among clinicians, basic researchers, politicians, and patients, and he can move very gracefully between all those constituencies."

DEATHS

In harmony. Seismologist Keiiti Aki never strayed far from his favorite subject. After a 38-year-long research career in the United States, he retired to Réunion Island, east of Madagascar, home of an active volcano that generated many earthquakes for him to study. On 17 May he died at the age of 75.

Although his work on the concept of seismic moment as a measure of earthquake magnitude may be his best-known contribution, Aki applied a quantitative rigor to understanding everything from great earthquakes to seismic imaging of Earth's interior and the crackle of moving magma.

"He had a profound view of the Earth," says seismologist Thomas Jordan of the University of Southern California in Los Angeles, "and a deep respect for the harmony and poetry of the natural world. Much of what we know about large earthquakes follows from his work."

AWARDS

Building capacity. An Indian physicist and a Brazilian biologist are the inaugural winners of a prize for scientists in developing countries.

Tiruppattur Ramakrishnan, a theoretical physicist at Banaras Hindu University in Varanasi, India, will receive the Trieste Prize from the Academy of Sciences for the Developing World (formerly the Third World Academy of Sciences) for his work on phase transitions and the localization of electrons in disordered systems. Pharmacologist Sergio Henrique Ferreira of the Medical School of Ribeirão Preto in Brazil is being honored for research that has led to development of new classes of antihypertensive drugs and analgesics. Each winner receives $50,000.

Future awards, made annually, will honor work in a variety of fields, including math and agricultural sciences.

CREDITS (TOP TO BOTTOM): MACIAS STUDIOS; SOURCE: JOEL KUPERSMITH; SOURCE: DAVID KHAYAT; COURTESY OF USC COLLEGE OF LETTERS, ARTS AND SCIENCES/SOUTHERN CALIFORNIA EARTHQUAKE CENTER

The Question of Forbidden Knowledge

THE POLICY FORUM "FORBIDDEN KNOWledge" by J. Kempner *et al.* (11 Feb., p. 854) erroneously conflates three distinct issues: (i) ethical questions surrounding certain types of practical experimentation; (ii) deeper epistemological ethics of scientific discovery and of knowledge itself; and (iii) the ethics of sharing or publishing existing scientific knowledge.

> **"[V]astly more dangerous to free scientific inquiry is the pressure exerted by funding sources, public and private, to steer the direction of research and to suppress ("forbid") critical scientific findings."**
>
> –WILLIAMSON

The first is a debate in progress. The second is scarcely a living issue at all and simply muddies the water by raising the old obscurationist bogeyman. The third evokes different immediate concerns that the authors fail to address.

Today, one would be hard pressed to find a pope, preacher, or president willing to seriously argue that "there are some things we must not know." Yet we agree that there are some methods we must not use—e.g., human experimentation without informed consent. Serious ethical debate on human cloning and stem cell research is not at all about "forbidden knowledge," but instead addresses important and still-unresolved material issues of "unacceptable means."

However, vastly more dangerous to free scientific inquiry is the pressure exerted by funding sources, public and private, to steer the direction of research and to suppress ("forbid") critical scientific findings. When religious groups decry the publication of research data about human sexuality, that is pitiful. When corporations withhold adverse information on their products, when they concoct or quash studies to prevent or conceal negative findings that could endanger their profits, that is criminal and too often deadly. The latter is the real "forbidden knowledge" problem that ought to concern ethical scientists far more immediately than should the ancient canard of obscurationism.

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IN THEIR POLICY FORUM "FORBIDDEN knowledge" (11 Feb., p. 854), J. Kempner *et al.* discuss the enduring concept that there exist ideas or bodies of knowledge that humanity should not know. However, some of their examples reveal a problem common to many ethics-related propositions: the confusion between means and ends (*1*). For example, they cite the Nazis' forced human experimentation programs during World War II that investigated treatment modalities for battlefield traumas, hypothermia, malaria, and so forth. The end knowledge from these trials is purported to be tainted, precisely because of the way it was obtained. In actuality, here it is strictly the means that are forbidden, not the end. The distinction is fundamentally important. One can readily pursue other avenues of inquiry as means to the same desirable end, for example, computer simulation, animal modeling, or informed-consent trials (*2*–*4*). None of these approaches raise the same level of ethical concern.

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THE POLICY FORUM "FORBIDDEN KNOWLedge" by J. Kempner *et al.* (11 Feb., p. 854) describes interviewees' reports of constraints on their research. I would like to call attention to the particular difficulties faced by the social sciences, including the related issue of the destruction of irreplaceable data from past research. For example, under the Native American Grave Protection and Repatriation Act (NAGPRA), large numbers of research specimens, assembled over more than a century—burial goods and skeletal remains from numerous research collections—have been returned to Native American tribes for their burial and permanent destruction (*1*). Similarly, the Smithsonian Institution destroyed, at the behest of Yale University, thousands of somatotype photos and negatives of Yale students (*2*), of the sort used by William H. Sheldon in compiling his *Atlas of Men* (*3*).

Part of the special difficulties the social sciences face comes from their inherently controversial subject matter. But another part, unique to them, arises because social scientists are often members of the social groups they study or participants in the phenomena they investigate. This leads to pressures against telling the truth that arise from their divided loyalties. For example, historians at the Smithsonian Institution were prevented from putting on an exhibit examining the dropping of the atomic bomb on Hiroshima, and the scaled-down Enola Gay exhibit contained inaccuracies and distortions (*4*). Whether the loyalty is to their country, gender, ethnic group, "race," religion, employer, or other group, if social scientists study it, they have a duty to describe it, warts and all, to the best of their abilities (*5*).

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Response

FORBIDDEN KNOWLEDGE, AS DISCUSSED IN the philosophical literature, is understood both as a deeply rooted historical belief that inquiry needs limits and as a social category whose content is closely aligned to time and culture. What is forbidden in one culture—e.g., fetal tissue research—is allowed in another. What was once acceptable—e.g., experimenting on prisoners—is now outlawed. Restrictions on inquiry can change depending on the strength of various interests, whether scientific, corporate, political, cultural, or religious.

Williamson and Wendle protest our definition of forbidden knowledge, arguing that we have conflated epistemological, methodological, and practical issues. Our definition of forbidden knowledge is intentionally broad and designed to capture any "knowledge" that is suppressed because that knowledge, or the methods needed to obtain it, is viewed as dangerous or subversive. If, as Wendl suggests, new technologies allow the safe and ethical collection of previously forbidden knowledge, then that knowledge would no longer meet our criteria. Defining the boundaries of forbidden knowledge, as with other issues in science, is a continuing process.

We agree with Williamson that the corporate suppression, distortion, and manipulation of findings ought to be addressed by legislators, university administrators, and scientists. We disagree, however, that no civic or religious leaders believe that "there are some things we must not know." For example, the

President's Bioethics Advisory Council recently concluded (albeit not unanimously) that human cloning presents a dangerously radical form of human procreation that would undermine the way we think about identity, family, and the production of life (*1*). Because of similar concerns, the U.S. House of Representatives passed a bill (by a vote of 241 to 155) that would criminally punish experimentation with human clones aimed not only at reproduction, but at developing therapies for human diseases (and went so far as to ban importation of drugs made using cloned cells in other countries) (*2*).

> **"[T]here needs to be open discussion about the causes and acceptability of, and policy responses to, practices that limit scientific inquiry."**
>
> –KEMPNER ET AL.

Fish's concern is grounded in the false dichotomy between the so-called objective "hard" sciences and the subjective "soft" sciences. A large body of research—including our own—has demonstrated that culture shapes how scientists decide to collect, use, interpret, and publish data (*3*–*5*). Many social scientists even argue that their group membership provides valuable interpretive insight (*6*, *7*). Fish also decries the destruction of data. Our study showed that researchers sometimes feel compelled to limit their inquiry or dissemination of data. They do this for ethical reasons, but also to avoid unwanted controversy.

We believe that there needs to be open discussion about the causes and acceptability of, and policy responses to, practices that limit scientific inquiry.

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The Problems of Radiocarbon Dating

IN THEIR PERSPECTIVE "THE BOON AND BANE of radiocarbon dating" (21 Jan., p. 362), T. P. Guilderson *et al.* raise some important issues in radiocarbon (^{14}C) dating. They discuss the problems of calibrating ^{14}C dates that fall within "age plateaus" of the ^{14}C calibration curve and conclude that "Far too often, the interpretations of leads, lags, or synchronicity of paleoclimate records are not fully supported by the radiocarbon chronology." Although we agree with their statements Guilderson *et al.* in our opinion, fail to highlight three vital points.

First, it is very rare nowadays to use one single date for inferring age models in archaeological and paleoclimate contexts. Robust Bayesian statistical techniques are available for handling sets of ^{14}C dates (*1*–*4*). Through careful and explicit use of statistics, the inherent uncertainties of isolated ^{14}C dates can be overcome, and the leads, lags, or synchronicity between different events can be properly identified.

Second, although they focus on the advantages of high-precision ^{14}C dates, Guilderson *et al.* do not mention that even a high-precision ^{14}C date is of very limited use if it is not accurate. Radiocarbon dates often need adjustments up to several ^{14}C "centuries" for age offsets that are only approximately known and that could vary with time [e.g., (*5*)]. Moreover, owing to contamination or handling errors, one in every 10 to 20 ^{14}C dates appears to be "outlying" (*6*). The outlier problem is far from trivial: In many studies, individual ^{14}C dates are removed manually and heuristically because they do not appear to fit the other data and the model applied (e.g., assumed chronological order of dates). Although perhaps not used widely enough, statistical techniques do exist to systematically handle both age offsets and outliers (*1*–*4*).

Finally, we note that Guilderson *et al.* work with 1σ (68%) calibrated ranges. Although this could be a reasonable approach for data with a Gaussian probability distribution, this is not the case for ^{14}C dates, where calibration nearly always gives rise to highly multimodal distributions. Reducing such distributions to mere 1σ ranges can result in a considerable loss of information. We therefore argue that, at least for ^{14}C dates of centennial-scale studies, their probability distributions on the calendar scale should be provided.

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Response

IN PRINCIPLE, WE AGREE WITH BLAAUW AND Christen's comments and provide the following clarifications. In our Perspective, we used variable ^{14}C dating precision (±40 versus ±15 years) as a vehicle to promote the thoughtful consideration of achievable final dating accuracy for routine samples, including ^{14}C-calendar calibration contributions. In hindsight, we perhaps should have stressed that the commonly used 68% (or 95%) calibrated age ranges are derived from the calibration probability distributions, providing a condensed version of the information available from the source distributions. We used the simplification provided by the 68% calibrated age ranges for the purposes of estimating calibration uncertainty and hence achievable final dating accuracy over the thousands of years covered by the international calibration standard IntCal98. We agree with Blaauw and Christen that it would be desirable to present the probability distributions themselves when such information is of significance in particular studies.

Many studies (including marine-based calibration data) inherently or explicitly assume age offset constancy through time. This assumption almost certainly does not reflect reality going back thousands of years and through various climatic regimes. The synthetic calibrations that we performed are equivalent to ^{14}C dating well-preserved terrestrial macrofossils of known provenance and provide the "best case scenarios" for calibration purposes with respect to, for example, paleoclimate studies. In regard to the "outlier problem," full understanding of this issue requires in-depth consideration of the international intercomparison study that Blaauw and Christen cite. Such considerations suggest that simple statements like "one in every 10 to

Letters to the Editor

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20 ^{14}C dates appears to be 'outlying'" do not reflect the complexity of the results obtained in the intercomparison study (*1*). The results indicate that 14% of the 92 laboratories participating in the study were responsible for more than 60% of the outliers.

We suggested the use of a priori information to improve chronologies, which can take advantage of Bayesian statistical techniques [e.g., adopted by Bcal (*2*), mexcal (*3*), and OxCal (*4*) calibration programs] to increase the accuracy of calibrated ages through exclusion of unlikely calibrated age results on individual dates. Notwithstanding Blaauw and Christen's emphasis on the application of statistical methods, and although it may be rare nowadays for a single date to be used for inferring age-models, all too often the chronology for a project is underfunded or appropriate material is lacking, resulting in a handful of dates spread too far apart to benefit from statistical modeling. In terms of overall reliability of the final chronologies, the selection of the proper quality materials for dating and a clear understanding of the association of those materials with the events of interest is of paramount importance—certainly much more important than any subsequent considerations of the optimal statistical methods to use in calibrating the ^{14}C dates.

Subsequent to publication of our Perspective, the international radiocarbon community has replaced IntCal98 with IntCal04 (*5*, *6*). We strongly recommend that the scientific community take advantage of IntCal04 for all appropriate calibration work.

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TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Thymic Origin of Intestinal αβ T Cells Revealed by Fate Mapping of RORγt$^+$ Cells"

Benedita Rocha

CD8αα intraepithelial gut T lymphocytes (IELs) differ from other T cells and have been proposed to differentiate from local precursors. Eberl and Littman (Reports, 9 July 2004, p. 248), however, argued that IELs expressing the αβ T cell receptor originate from CD4$^+$CD8$^+$ thymocytes. We find their experiments inconclusive and their interpretations inconsistent with previously published data.

Full text at www.sciencemag.org/cgi/content/full/308/5728/1553a

RESPONSE TO COMMENT ON "Thymic Origin of Intestinal αβ T Cells Revealed by Fate Mapping of RORγt$^+$ Cells"

Gérard Eberl and Dan R. Littman

We maintain that intraepithelial intestinal T lymphocytes expressing the αβ T cell receptor are most likely derived from thymocytes and that cells previously identified as local precursors instead function as inducers of lymphoid follicles. In contrast to earlier studies involving cell transfers into athymic lymphopenic mice, our interpretations are based on nonperturbing genetic approaches.

Full text at www.sciencemag.org/cgi/content/full/308/5728/1553b

HUMAN GENETICS

Lessons from the HGDP?

Henry T. Greely

It is probably a useful thing for a researcher, every once in a while, to become a research subject—useful, but not entirely comfortable. Jenny Reardon's *Race to the Finish: Identity and Governance in an Age of Genomics* is built around an analysis of the Human Genome Diversity Project (HGDP). As someone associated with that project since 1993 (and one of the author's interviewees), reading the book was like viewing familiar scenes through someone else's glasses. The people and places were recognizable, but the experience was unsettling.

Proposed by a group of geneticists in 1991, the HGDP was intended to create a resource for studying the breadth of genetic variation in our species. By September 1993, it had defined its initial goals: to collect DNA samples from 500 human populations around the world, to make this DNA available to qualified researchers, to analyze it using a common set of markers, and to put the resulting data in an accessible database. The project hoped to accomplish these goals in five to seven years at a total cost of $25 to $35 million. As it happened, the HGDP collected more controversy than DNA and never received substantial funding. To date, its major scientific accomplishment is the gathering of just over 1000 human cell lines at the Fondation Jean Dausset–Centre d'Etude du Polymorphisme Humain in Paris, most of them originally collected under other auspices. DNA from these cell lines is available to qualified researchers and has been used in valuable research (*1*). Some would also count the "Model Ethical Protocol for Collecting DNA Samples" crafted by the HGDP's North American Regional Committee (*2*) as a substantial accomplishment.

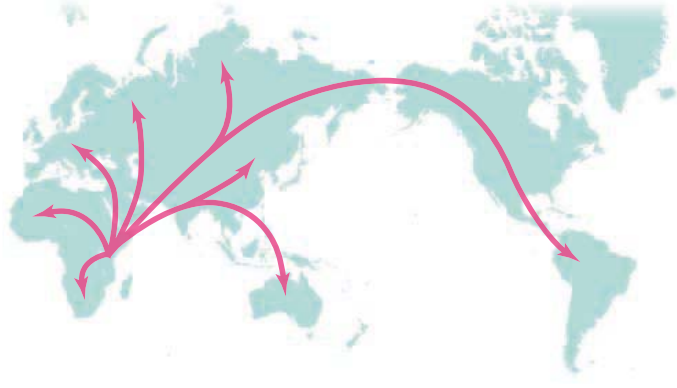
The book begins with an introductory chapter that will probably most interest those within the field of science studies. It positions the HGDP as an example of "co-production" of scientific and social understandings. In the following two chapters, Reardon (a researcher at Duke University's Institute for Genome Sciences and Policy) describes how anthropologists and geneticists variously remade the idea of race after—and because of—World War II. The next three chapters examine the history of the HGDP in light of particular themes: its encounters with anthropologists, its attempt to create a "group consent" requirement for human population genetics research, and its efforts to encourage members of the study populations to participate in the project. A final chapter draws some lessons for similar future efforts.

Race to the Finish
Identity and Governance in an Age of Genomics
by Jenny Reardon

Princeton University Press, Princeton, NJ, 2005. 251 pp. $55, £35.95. ISBN 0-691-11856-6. Paper, $17.95, £11.95. ISBN: 0-691-11857-4. In-formation Series.

Reardon has written a valuable book. As a participant in the events recounted, I would—given unlimited space—challenge many of her interpretations, but I have only one major complaint. She accuses the HGDP of being unconscious of its own political nature while devaluing all opposition as politically inspired. Reardon never considers that some of the opposition was at least partially politically motivated, seeking to put the HGDP into no-win situations. If it did not consult with activists from indigenous groups, it was wrongly ignoring them; if it did consult with them, it was wrongly trying to co-opt them. Neither the HGDP nor its critics were completely political or completely apolitical, something many HGDP members recognized.

I have three broader comments. First, the book stresses that science is situated in its society and that the HGDP, in particular, was situated in the geneticists' post–World War II vision of race. Of course, Reardon's book itself is situated in its time and place, one that infuses her interpretation of the HGDP with race to a much greater extent than the HGDP ever did—note the title, ***Race*** *to the Finish*. HGDP planners did not ignore race; they worried that the project's results might be misused to abet racism and hoped that its findings would undermine racism. But they did not use the term "populations" as code for "race." Although they hoped the project would help illuminate ancient human migrations and the consequent creation of broad ancestral groupings that might be talked about as races, they also wanted to help explain smaller and more recent questions such as the origins of Basque and Plains Indian populations and the spread of Bantu-language speakers. Perhaps the HGDP paid too little attention to race; perhaps the author pays it too much.



Paths to human diversity.

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Second, the project itself contained diversity. Different organizers argued for different things and even read agreed-upon documents differently. And individuals could and did have more than one motive. For example, Reardon argues that group consent was adopted not because it was ethical but because it had instrumental value for the HGDP. Of course, it could be—and many thought it was—both ethical and useful.

Third, the book illustrates the advantages one who observes has over one who acts. At one point, Reardon contends that the HGDP needed to resolve "deeply entrenched and contested questions about the nature of knowledge about the human, and the relationship of this knowledge to modern techniques of power." At another, she complains that the project's ethical guidelines "cordoned off the complex political questions about North/South relations and indigenous rights." Even if the HGDP organizers had agreed, I cannot see how they could have said, "Let's stop the project until we solve these intractable issues." Anyone trying to act deals with an uncertain world where trade-offs, guesses, and least-bad decisions are essential; subsequent scholarly critics do not. (The same applies, of course, to book writers and book reviewers.)

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The most direct reason for the HGDP's lack of success was lack of funding. In spite of some loud criticism, it seems likely that several hundred of the world's many populations would have participated. The whole project would have taken only a small investment by the U.S. National Institutes of Health. Did NIH decide not to fund the HGDP because of concerns about the reactions of anthropologists or indigenous peoples, because of concerns about the program's leadership or structure, merely because of other priorities, or for other reasons? I was disappointed that Reardon sheds little light on this question.

The NIH and others are currently sponsoring another approach to human genomic variation through the International Haplotype Map Project. The National Geographic Society has just announced its own "Genographic" project that seeks, in essence, to carry out the goals of the HGDP with private funding. Reardon is clearly correct that these projects, and others, will face many of the issues that dogged the HGDP. To the organizers of those projects, as well as to a broader audience, I strongly recommend *Race to the Finish*. Although Reardon does not provide "the" story of the HGDP, she offers a useful story of the problems that effort faced.

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10.1126/science.1111130

MEDICINE

Addressing Pregnancy Loss

John Aplin

The author of *Coming to Term*, a science journalist and correspondent for *Science*, had good reason to look into the causes of pregnancy failure. Jon Cohen's partner suffered four consecutive miscarriages in her late thirties and early forties. Cohen explores the many strands of miscarriage research from the mid 20th century to the present; he talked to couples with tragic and, in some cases, extraordinary histories of repeated pregnancy failure; and he got to know doctors who do their best to help. His absorbing book integrates the subjective experience of an aspiring father cast onto the emotional roller coaster of repeated pregnancy loss with an intellectual journey through the history of reproductive immunology, and it successfully illuminates the rationale behind treatments for multiple miscarriage, some of which seem situated between the bold and the scary.

Coming to Term
Uncovering the Truth About Miscarriage
by Jon Cohen
Houghton Mifflin, Boston, 2005. 288 pp. $24. ISBN 0-618-27724-2.

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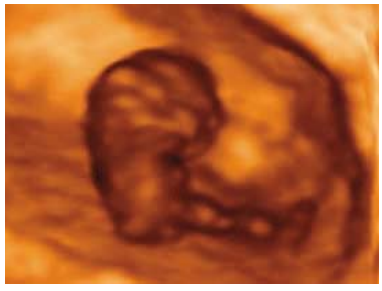
The field of reproductive immunology was spawned by Peter Medawar's musings, in a notable 1952 lecture, on the question of why the fetus is not rejected by the mother's immune system. One possibility was that the mother temporarily tolerates antigen from the father, but this notion was discarded when grafts of paternal skin were rejected by pregnant females. Later, it was realized that the major histocompatibility complex antigens are absent from the trophoblast (the outermost cells of the placenta, genetically and developmentally of fetal origin) and that the lack of these immune markers is one key to the survival of the fetus. Nonetheless, an immune dialogue occurs between mother and fetus; indeed, immune recognition is critical for successful pregnancy outcome.



Over most hurdles. More than 90 percent of miscarriages occur by the eighth week, the age of the fetus in this colored 3D ultrasound scan.

As Cohen's account indicates, miscarriages also occur for non-immunological reasons. A staggeringly high proportion—in fact, a majority—of embryos produced by otherwise normal human couples are chromosomally abnormal. Though many of these embryos do not pass early developmental milestones and go unrecognized, others develop to form normal-seeming blastocysts, many of which implant only to miscarry during the first trimester. This complicates the interpretation of data arising from attempts to treat miscarriage and helps explain why it has been so difficult to make sense of the literature in this area. In particular, when examining the effectiveness of a treatment for a problem in the uterus, one must exclude abnormal embryos, which a normal uterus is presumably programmed to reject.

Unfortunately we are in the dark about both the mechanisms that underlie the loss of normal embryos and the extent to which immune recognition contributes to the "normal" rejection of an abnormal embryo. The latter is not a trivial problem; a placenta may survive in utero for a substantial time, apparently unaffected, after the death of the fetus to which it is connected. Most current diagnostic tests for miscarriage syndromes are measurement surrogates for defects that compromise pregnancy for reasons that are neither identified nor understood.

Miscarriage places unusual pressures on the doctor-patient relationship. Cohen notes that after three consecutive spontaneous miscarriages, there is still a 70% chance women will, with no treatment, carry their next pregnancy to term. Furthermore, trials have shown consistently that "tender-loving care" improves outcomes in the absence of any other intervention. Formerly desperate people with healthy new babies ascribe credit to doctors who have administered one or another therapy. The doctors—mostly sincere, patient, and long-suffering—feel only too keenly the lack of a magic formula for pregnancy success. A minority have become wealthy touting unproven interventions. But unbiased trials have far too often suffered delays as well-informed, aging couples do their utmost to avoid being randomized into a control group. The author's survey of treatments is intended to help readers evaluate the options.

There are rapid current advances in ovarian biology, so the fact that most chromosomally defective embryos arise from abnormal oocytes rather than sperm is, in a way, good news for miscarriage research. Although there are some major resourcing issues to be addressed (no public health system will fund routine preimplantation genetic diagnosis for couples undergoing in vitro fertilization), stem cell biology and emerging reproductive technologies will yield improved understanding of the factors that lead to production of abnormal oocytes. Interventions made possible by the affluence of professional would-be parents in the developed world may, if applied in properly designed trials and in partnership with enlightened publicly funded basic research, give rise to a better understanding of the fascinating question of why our species is so surprisingly inefficient in its reproductive efforts. They may also provide therapies targeted to help people for whom full-term pregnancy just does not come easily. Meanwhile, Cohen's book provides a valuable resource for couples seeking help in deciding a course of action in the face of difficulties with starting a family. For fertility researchers and practitioners, it offers an engaging account of a complex, important, and poorly understood problem.

10.1126/science.1114723

PUBLIC HEALTH

Pharmacist Refusals: A Threat to Women's Health

Marcia D. Greenberger and Rachel Vogelstein

Pharmacist refusals to fill prescriptions for birth control based on personal beliefs have been increasingly reported around the world. In the United States, reports of pharmacist refusals have surfaced in over a dozen states. These refusals have occurred at major drugstore chains like CVS and Walgreens and have affected everyone from rape survivors in search of emergency contraception to married mothers needing birth control pills. Pharmacists who refuse to dispense also often have refused to transfer a woman's prescription to another pharmacist or to refer her to another pharmacy. Other pharmacists have confiscated prescriptions, misled women about availability of drugs, lectured women about morality, or delayed access to drugs until they are no longer effective.

Pharmacist refusal incidents have also been reported in other countries. For example, a pharmacist at a popular London pharmacy chain recently refused to fill a woman's prescription for emergency contraception (EC), or the "morning-after pill," due to religious beliefs; two pharmacists refused to fill contraceptive prescriptions for women at a pharmacy in Salleboeuf, France; and in the small country town of Merriwa, Australia, the local pharmacist refuses to stock EC altogether (*1*–*3*). Pharmacists for Life International, a group refusing to fill prescriptions for contraception, currently claims to have over 1600 members worldwide and represents members in 23 countries (*4*).

Pharmacist refusals can have devastating consequences for women's health. Access to contraception is critical to preventing unwanted pregnancies and to enabling women to control the timing and spacing of their pregnancies. Without contraception, the average woman would bear between 12 and 15 children in her lifetime. For some women, pregnancy can entail great health risks and even life-endangerment. Also, women rely on prescription contraceptives for a range of medical reasons in addition to birth control, such as amenorrhea, dysmenorrhea, and endometriosis. Refusals to fill prescriptions for EC (a form of contraception approved by the U.S. Food and Drug Administration and relied on worldwide) are particularly burdensome, as EC is an extremely time-sensitive drug. EC is most effective if used within the first 12 to 24 hours after contraceptive failure, unprotected sex, or sexual assault. If not secured in a timely manner, this drug is useless. Rural and low-income women, as well as survivors of sexual assault, are at particular risk of harm.

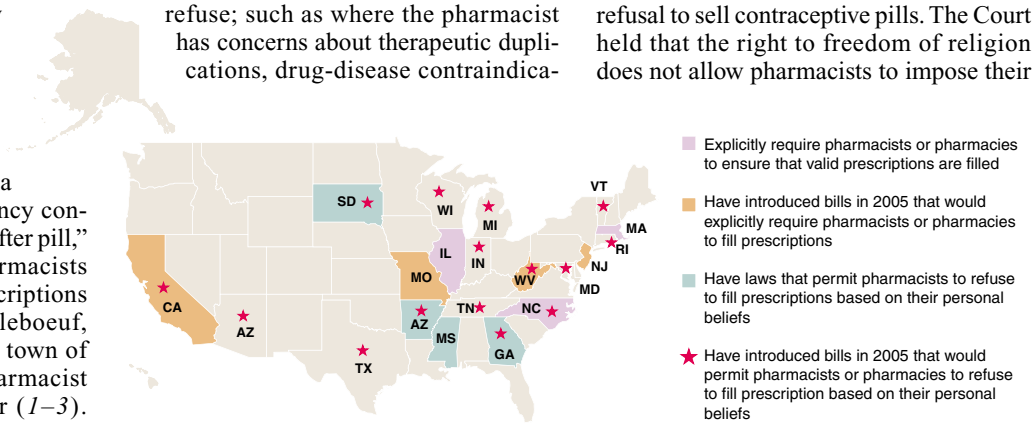
In the United States, most states have an implied duty to dispense. Personal beliefs are omitted from the enumerated instances where pharmacists are authorized to refuse; such as where the pharmacist has concerns about therapeutic duplications, drug-disease contraindications, drug interactions, incorrect dosage, or drug abuse. In New Hampshire, the pharmacy regulations' Code of Ethics states that a pharmacist shall "[h]old the health and safety of patients to be of first consideration and render to each patient the full measure of his/her ability as an essential health practitioner" (*5*). Pharmacists who refuse to fill valid prescriptions based on personal beliefs do not hold patient health and safety as their first consideration.

Illinois explicitly charges pharmacies with a duty to ensure that women's prescriptions for birth control are filled without delay or interference (*6*). Massachusetts and North Carolina have interpreted their laws to ensure that women's access to medication is not impeded by pharmacists' personal beliefs (*7*, *8*). However, Arkansas, Georgia, Mississippi, and South Dakota explicitly grant pharmacists the right to refuse to dispense prescriptions for birth control based on personal beliefs (*9*).

In addition, a small number of administrative and judicial bodies have considered challenges to pharmacist refusals. In the United States, the Wisconsin pharmacy board found that a pharmacist's failure to transfer a birth control prescription fell below the expected standard of care and constituted a danger to the health and welfare of the patient. The board formally reprimanded the pharmacist for his actions, charged him with the $20,000 cost of adjudication, and conditioned his license on provision of proper notification to his employer about anticipated refusals and his assurances about steps he will take to protect patient access to medication (*10*).

Outside of the United States, the European Court of Human Rights rejected an appeal of a conviction of pharmacists under the French consumer code for a refusal to sell contraceptive pills. The Court held that the right to freedom of religion does not allow pharmacists to impose their beliefs on others, so long as the sale of contraceptives is legal (*2*).

Some have questioned how such rules comport with the treatment of other medical professionals. In general, medical professionals have a duty to treat patients, with only limited exceptions. The majority of refusal laws apply to doctors and nurses and are limited to abortion services. Allowing pharmacists to refuse to dispense prescriptions for contraception would dramatically expand the universe of permissible refusals. Moreover, unlike doctors and nurses, pharmacists do not select or administer treatments or perform procedures. Therefore, pharmacists' involvement is not as direct, nor would patients'



Laws and bills governing pharmacist refusals.

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safety be potentially compromised in the same way as would be the case if a doctor or nurse were forced to perform a procedure that they personally oppose.

Since 1997, 28 states have introduced legislation that would permit pharmacists to refuse to dispense, and sometimes to refer or transfer, drugs on the basis of moral or religious grounds. Fifteen states have introduced such bills in the 2005 legislative session alone (see figure, p. 1557); while some are specific to contraception, others apply to all medication. These bills have implications for future refusals to fill prescriptions, such as in HIV regimens or treatments derived from embryonic stem cell research. On the other hand, bills have been introduced in four state legislatures and the U.S. Congress that would require pharmacists or pharmacies either to fill prescriptions for contraception or ensure that women have timely access to prescription medication in their pharmacies.

Some professional and medical associations have issued guidelines that protect women against pharmacist refusals. Value VIII of the *Code of Ethics* of the College of Pharmacists of British Columbia requires pharmacists to ensure "continuity of care in the event of … conflict with moral beliefs" (*11*). It permits pharmacists to refuse to dispense prescriptions based on moral beliefs, but only if there is another pharmacist "within a reasonable distance or available within a reasonable time willing to provide the service."

In the United States, several associations have issued similar, although not legally binding, policies. The American Public Health Association states that "[h]ealth systems are urged to establish protocols to ensure that a patient is not denied timely access to EC based on moral or religious objections of a health care provider" (*12*). The American Medical Women's Association has stated that "pharmacies should guarantee seamless delivery, without delay (within the standard practice for ordering), judgment, or other interference, of all contraceptive drugs and devices lawfully prescribed by a physician" (*13*).

The American Pharmacists Association (APhA) articulates a standard of professionalism in its *Code of Ethics* that is not legally binding. It mandates that pharmacists place "concern for the well-being of the patient at the center of professional practice" (*14*). The code also emphasizes that pharmacists are "dedicated to protecting the dignity of the patient" and must "respect personal and cultural differences …" (*14*). This language precludes refusals, lectures, and other barriers erected by pharmacists who disagree with a woman's decision, made in consultation with her health-care provider, to use birth control. Some state pharmacy associations have similar codes.

However, the APhA has another policy that conflicts with these principles. It allows for refusals based on personal beliefs, as long as pharmacists refer prescriptions to another pharmacist or pharmacy (*15*). The APhA has not formally explained how to square this policy with its ethical principles of patient-protective care, let alone with state laws and regulations.

Recommendations

Women must be provided timely access to prescription medication. One solution is to require pharmacists to dispense all drugs despite their personal beliefs, in line with their professional duties and ethical obligations. Another solution is to shift the duty to fill from pharmacists onto pharmacies. Under this approach, pharmacies would be charged with ensuring that prescriptions for all drugs are filled without delay or other interference. Such a requirement would allow pharmacies to make arrangements to accommodate the personal beliefs of individual pharmacists. However, active obstruction by pharmacists of women's access to prescription medication—such as withholding or delaying prescriptions or providing misinformation—should be deemed unethical or unprofessional conduct subject to legal sanction.

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10.1126/science.1114466

PUBLIC HEALTH

Conscientious Objection and the Pharmacist

Henri R. Manasse Jr.

The recent deluge of media attention about conscientious objection and the role of the pharmacist in helping patients obtain medications that some consider morally objectionable (e.g., the "morning-after pill") presents an opportunity to find common ground in what has become a highly charged public debate. State legislators and members of the U.S. Congress have even jumped into the fray with quickly drafted legislation that fails to address the heart of the problem (*1*).

On its face, the issue appears to be simple and straightforward: Should pharmacists who object to the use of certain reproductive medications be allowed to remain true to their beliefs and conscience within the context of their daily work? Should female patients who are seeking these medications be allowed unfettered access to them? As both arguments are logical when viewed from their respective points of reference, the answer on both counts is "yes." The issue is how to balance these divergent but important needs while honoring the humanity of both parties.

The egregious actions of a small number of pharmacists who have obstructed access to the medicines in question are clearly unprofessional and unacceptable. Indeed, the "Code of Ethics for Pharmacists" (*2*) states that a pharmacist must respect the "covenantal relationship between the patient and pharmacist" and the "autonomy and dignity of each patient."

What has been lost in the media coverage of and political dialogue about this issue are the nuances and implications of conscientious objection. Like their physician and nurse colleagues, pharmacists routinely operate within both professional and personal ethical frameworks (*3*).

On a personal level, pharmacists have the same rights as their fellow health-care

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colleagues. Like a surgeon who refuses to perform abortions because of a personal moral objection or a nurse who believes that turning off a patient's respirator would contradict her beliefs on the sanctity of life, pharmacists must be allowed to be true to their own belief systems as they practice their profession.

This does not mean that pharmacists should be allowed to impose their personal morals on patients under their care. As with physicians and nurses, it simply means that pharmacists must maintain the right to "step away" from the offending activity and should refer the patient in question to another pharmacist who can dispense the prescription (*3*).

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Pharmacists, physicians, and patients best work together as a tripartite team, with each party maintaining good communication with the others to optimize patient care (*4*). When a pharmacist is concerned about the dose or choice of a prescribed medication for therapeutic reasons, it is well accepted that he or she contacts the prescriber. Objections based on moral considerations should be handled the same way and, in fact, are in many practice environments. Effective communication between prescriber, patient, and pharmacist can assure that patients always have access to the medicine they need.

Unfortunately, the actions of a few extreme individuals have been met with equally extreme governmental edicts and legislation. In Illinois, Governor R. R. Blagojevich recently issued an emergency rule requiring pharmacies that stock the morning-after pill to dispense it without delay. This rule is not the appropriate answer to the dilemma we face.

The difficulty with this rule, and others like it, is the phrase "without delay." That is because pharmacists routinely work with physicians to clarify or alter medication orders that could potentially cause harm to patients. This critical consultation takes time. As medication-use experts who have had a minimum of 6 years of specialized education about today's powerful and complex drug products, pharmacists often advise prescribers on new and effective therapies and the selection and administration of medications.

Pharmacists also perform critical quality checks to detect and prevent harmful drug interactions, adverse reactions, and mistakes—all essential patient-safety activities that would be thwarted by legislation requiring a blanket mandate to fill a prescription order without delay. If policymakers desire to make the morning-after pill available without the benefit of the expertise of pharmacists, then they should advocate over-the-counter sale.

Pending legislation and other measures to deal with this controversy are also afoot in many states and in Congress, including the Prevention First Act (*5*). In some states, such as Arizona (S.B. 1485), Rhode Island (H 5085), Michigan (H.B. 4741), and Texas (H.B. 2061), legislation is being proposed that would allow pharmacists to refuse to dispense certain reproductive drugs on moral grounds (*6*–*9*). In other states, like New York (A. 116), Massachusetts (S.B. 546), and Colorado (H.B. 1042), there are proposals or newly enacted laws requiring hospitals to offer the morning-after pill to rape victims or allowing certain pharmacists to sell it without a prescription (*10*–*12*).

Clearly, the issue of how the pharmacy profession will balance the dispensing of certain controversial medications with pharmacists' ability to exercise their own personal beliefs is here to stay. Pharmacists must balance their right to conscientious objection while respecting the patient's right to access FDA-approved therapies.

In recent years, the pharmacy profession has devoted much time and thought to the issue of conscientious objection. The American Society of Health-System Pharmacists (ASHP) first adopted a professional policy on this issue in 1998 and reaffirmed it in 2002. It recognizes a pharmacist's right of objection and concurrently supports the establishment of systems to protect the patient's right to obtain legally prescribed and medically indicated treatments (*13*). This policy states that "pharmacists must retain their right to participate or not in morally, religiously, or ethically troubling therapies." ASHP also has in place an official "Statement on Pharmacist's Decision-Making on Assisted Suicide" (*14*), which establishes a framework for pharmacist participation in the legal and ethical debate about the appropriate care of patients at the end of life.

As the nation continues to debate this issue, there are a variety of solutions that can address the problem before us: (i) Pharmacies must be appropriately staffed so that if one practitioner chooses to step away, another pharmacist can assist the patient; (ii) pharmacies should have policies in place to redirect patients to alternative sources if the facility either does not stock or does not support dispensing the medications in question; (iii) pharmacists should work with physicians and other prescribers to establish alternative dispensing methods for medications that have been identified as controversial; (iv) the availability of a national toll-free number to help women find access to outlets for emergency contraceptives (888-NOT-2-LATE) (*15*) should be better publicized; and (v) physicians and pharmacists should work together to identify local pharmacies that provide these therapies.

Like all health-care professionals, pharmacists should consider the scope of their objection and comfort level in ensuring patient access before they ever begin to care for patients. Conflict between pharmacists and patients over potentially morally objectionable therapies is not a foregone conclusion. It is the exception to the millions of positive interactions among pharmacists and patients that occur every day in this country.

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10.1126/science.1114371

BIOMEDICINE

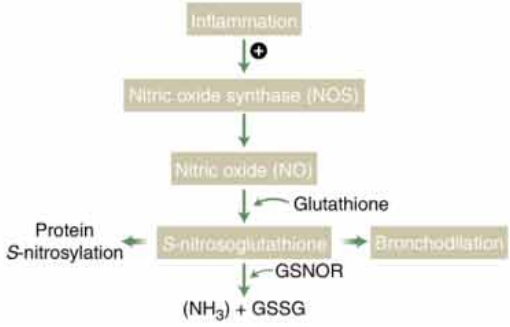
Asthmatics Breathe Easier When It's SNO-ing

Craig Gerard

Asthma is now recognized as an epidemic in the developed world, focusing attention on possible therapies. Although much has been learned over the last two decades about the pathogenesis of asthma, this complex disease resists magic-bullet therapies. In part, this resistance may be caused by the large number of genes that interact with underlying physiology and environmental factors to trigger disease. The chronic inflammation of the asthmatic lung results from an allergic reaction marked by elevated immunoglobulin E, mast cells and eosinophils, and cytokines such as interleukin-5 and interleukin-13. This chronic inflammation causes bouts of acute airway constriction. Eventually, asthmatic lungs show permanent changes: increased mucus cell mass, hypertrophy of the smooth muscle cells, and deposition of collagen just below the lining of the epithelial surface.

The airways of most asthmatics are hyperresponsive. Although in normal individuals, the cholinergic agonist methacholine causes bronchospasm, the asthmatic airway is even more susceptible to spasm induced by this drug—so much so that this response is used to diagnose asthma in the clinic.

The current mainstays of asthma therapy are directed at the lung inflammation and the consequent spasms. These include inhaled bronchodilators, anticholinergic drugs, anti-inflammatory drugs such as corticosteroids, and leukotriene antagonists. New therapeutic targets are not easy to identify because laboratory animals do not spontaneously develop asthma. An approximation of asthma has been developed in guinea pigs and rodents through immunization with antigens (ovalbumin or other asthma initiators such as *Aspergillus*, dust mites, etc.). Typically, these animals develop features that are reminiscent of the pathology of asthma: a T helper 2 (T_H2) T cell response, peripheral and airway eosinophila, T lymphocyte infiltration of the airways submucosa, mucus cell hyperplasia, and airway hyperresponsiveness to methacholine.



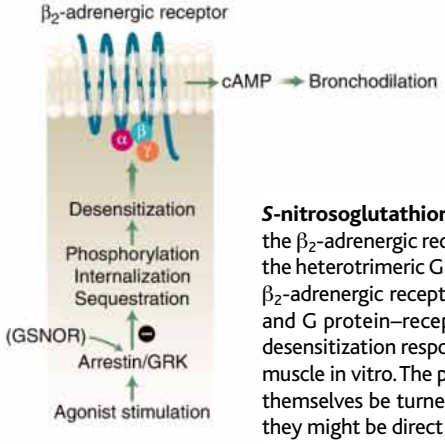
Production and fate of *S*-nitrosoglutathione. NO, produced by an NO synthase, reacts with glutathione to form *S*-nitrosoglutathione. This reactive molecule may directly dilate bronchi, cause protein *S*-nitrosylation (and affect signaling molecules, enzymes, transcription factors, etc.), or be degraded to oxidized glutathione (GSSG) and ammonias by *S*-nitrosoglutathione reductase (GSNOR).

The nitric oxide (NO) signaling system in the lung (*1*) has been implicated in asthma, because human asthmatics exhale abnormally high amounts of NO, and have the type 2 (inducible) nitric oxide synthase (NOS) in their airways and inflammatory cells. Nitric oxide is known to function as a smooth muscle relaxing factor and in fact was originally identified as the endothelial-derived relaxing factor, EDRF.

Three enzymes synthesize NO, converting L-arginine to NO and citrulline. Although each is constitutively expressed in lung epithelium, they were named according to the tissue where they were initially identified. Type 1 is neuronal NOS, type 2 is inducible NOS (expressed by many diverse cells), and type 3 is the endothelial NOS. Human asthmatic airways express type 2 NOS in their lungs, whereas healthy airways do not. Types 1 and 3 NOS are expressed in similar amounts in both asthmatic and normal airways. Gene knockouts in the mouse have been analyzed for each of these types of NOS: Animals lacking type 2 NOS did show somewhat less inflammation in the lung tissue sensitized and challenged with ovalbumin, but airway hyperresponsiveness persisted (*2*). Absence of type 3 NOS had no apparent effect on asthma responses. Knockout of type 1 NOS (the neuronal variant) produced a mild (~threefold) decrease in airway hyperresponsiveness, suggesting that this isoform was responsible for inflammation mediated by the bronchomotor neurons (*3*).

Thus, a link between NO and asthma has remained elusive. This has now changed with a report by Que *et al.* (*4*) on page 1618 in this issue. The authors describe a dehydrogenase involved in the metabolism of *S*-nitrosothiols that acts a modulator of allergic airway disease in mice and, by extension, perhaps humans. Their results link the NOS system to bronchial smooth muscle tone and to cholinergic responsiveness.

Nitric oxide, a highly reactive signaling molecule capable of several cytotoxic oxidative states, is quite short-lived. Thiols, largely in proteins, react with NO to yield *S*-nitrosothiols (SNOs), which are comparatively more stable. SNOs thus can serve as a storage depot for nitric oxide. In addition, some protein functions are changed by *S*-nitrosylation and small SNOs themselves can possess biological activity. The most common SNO in the airway is *S*-nitrosoglutathione. Glutathione concentrations in airway lining fluid are ~250 μM in normal individuals. In addition to its antioxidant properties, glutathione is



***S*-nitrosoglutathione directly affects smooth muscle tone.** Normally, the β_2-adrenergic receptor elevates cyclic AMP in smooth muscle through the heterotrimeric G protein complex; this relaxes the smooth muscle. The β_2-adrenergic receptor is desensitized through the actions of β-arrestins and G protein–receptor kinases (GRKs). In GSNOR knockout mice, the desensitization response (to isoproterenol) is not seen in tracheal smooth muscle in vitro. The proteins involved in the desensitization response may themselves be turned off by increased intracellular SNO. Hypothetically, they might be direct targets of protein *S*-nitrosylation.

CREDITS: PRESTON HUEY/*SCIENCE*

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a major "sink" for newly biosynthesized NO (see the first figure).

The steady-state level of *S*-nitrosoglutathione in the lung is the product of its biosynthesis and breakdown. The enzyme glutathione-dependent formaldehyde dehydrogenase functions as an *S*-nitrosoglutathione reductase (GSNOR) (*3*). Like the inducible type 2 NOS, levels of GSNOR are elevated in allergic asthmatics and in rodent models. If *S*-nitrosoglutathione is a potent bronchodilator, what is the consequence of increased activity of GSNOR? To find out, Que *et al*. deleted GSNOR in mice by homologous recombination. The basal bronchial tone of these mice was lower than in normal animals. When the mice were sensitized and challenged with ovalbumin, they developed acute inflammatory cell infiltration with lymphocytes and eosinophils, but not airway hyperresponsiveness. Increased levels of SNOs were found in lung secretions. Inhibition of type 2 NOS pharmacologically erased this phenotype. The authors' evidence suggests that endogenous SNOs regulate smooth muscle tone, and that insufficient SNOs promote airway hyperresponsiveness. Thus, inhibition of GSNOR may promote bronchial dilation.

Several key details require further study. First, the major source of GSNOR activity is intracellular. Enzyme in the fluid on the surface of allergic airways likely derives from necrotic or apoptotic cells. In asthmatic airways, where *S*-nitrosoglutathione is less abundant than normal, less NO is chemically incorporated and the remainder is exhaled as gas, thus accounting for the increased exhaled fraction of NO in asthmatics. Is GSNOR the cause of this? Or are oxidant and nitrosative stresses depleting the glutathione substrate?

Second, additional mechanisms regulate smooth muscle tone. Que *et al*. show that tracheal smooth muscle relaxes in vitro upon exposure to the bronchodilator isoproterenol, which acts on a G protein–coupled receptor that is coupled to adenylate cyclase. As with other such receptors, repeated signaling, in this case through exposure to isoproterenol, leads to desensitization (*5*); in GSNOR knockout mice, however, it did not. This result suggests that endogenous β-adrenergic agonists might have greater effects in relaxing smooth muscle in vivo. What might underlie this result? One well-described mechanism involves recruitment of the scaffolding β-arrestins and G protein–coupled receptor kinases to phosphorylate the intracellular segments of the G protein–coupled receptors (*6*), leading to desensitization. This mechanism suggests that intracellular kinases may be repressed by sulfhydryl nitrosylation; loss of GSNOR potentiates intracellular SNOs and antagonizes desensitization. Thus, GSNOR might regulate bronchomotor tone in vivo via a second pathway, that is, by potentiating signaling through G protein–coupled receptors (see the second figure).

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APPLIED PHYSICS

Nanoantennas for Light Emission

Jean-Jacques Greffet

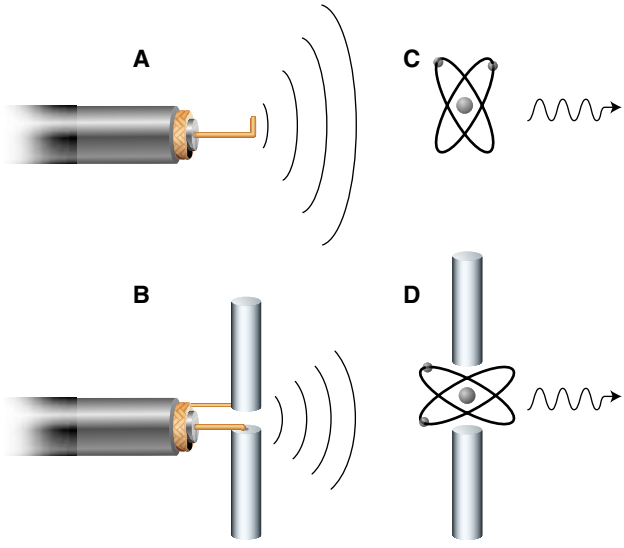
Nanoscience is by essence an interdisciplinary field in which traditional differences between disciplines vanish. On page 1607 of this issue, Mühlschlegel *et al*. (*1*) bridge the fields of electrical engineering and nanometer-scale optics to show that a thin, 100-nm-long metallic rod resonantly enhances light emission, just like an antenna enhances radio emission. The work paves the way for tailoring the light emission from nanometer-scale systems.

The emission of radio waves by antennas is discussed in many electrical engineering textbooks. On the other hand, emission of light by a single molecule is a cornerstone of nanooptics, with applications, for example, in quantum information processing or single-molecule spectroscopy. If the know-how from electrical engineering could be used for nanooptics applications, one might be able to enhance the optical emission rate and control the emission direction.

However, whereas for radio waves, a metal can be modeled by a perfect mirror, at optical frequencies the field can penetrate the material and can be absorbed. Moreover, absorption depends on the frequency of the light. Mühlschlegel *et al.* now show that ideas from electrical engineering can nevertheless be applied successfully to nanooptics.

A key issue in nanooptics is how the emission of light by a molecule can be modified. In a vacuum, an excited molecule releases its energy by emitting a photon at a rate that depends on its lifetime. Purcell showed in 1946 (*2*) that the amount of power emitted by an electromagnetic source depends on its environment. (This idea is commonly used for microwave emission, for example when a gun diode is placed in a resonant cavity connected to a waveguide to increase the emitted power.) In the same way, use of a wavelength-scale cavity modifies the emission rate of a single atom (*3*).

Another possibility for tailoring the optical emission is to create an environment that prevents the propagation of light. Because light is forbidden, an excited atom cannot generate a photon; the lifetime of the excited atom is thus increased. Light propagation can be prevented by using three-dimensionally periodic dielectric materials called photonic crystals (*4*). This



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Radio and optical antennas. The end of a coaxial wire (**A**) is a source of radio waves. Connecting the wire to an antenna (**B**) amplifies the radio emission and modifies its direction. Light emission can be modified in a similar way by placing a light source such as an atom (**C**) between two rods (**D**). In (C), the photon is emitted in almost any direction, whereas in (D), the emission direction is concentrated in directions perpendicular to the antenna.

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concept has been used to modify the lifetime of an excited state (*5*).

A third way of modifying the emission of radiation is routinely used in electrical engineering. Instead of inserting the source in a resonant cavity or in a photonic crystal, one can design an antenna (see the figure). An antenna modifies both the amount of energy emitted and the direction of emission. Let us consider a coaxial wire connected to a generator. Its end is a small dipolar antenna. By connecting the wire to a properly designed antenna, one can both increase the amount of radiated power and modify the angular emission pattern.

To understand the role of an antenna, consider the emission of acoustic waves by a guitar string. Without the guitar cavity, the string hardly emits any sound, showing clearly that the amount of emitted power strongly depends on the environment of the source. The antenna, like the guitar, can be viewed as an intermediate resonator that is efficiently coupled to both the source and the vacuum. From that point of view, there is not much difference between a microcavity and an antenna.

A key question for nanooptics is whether an antenna can be used to modify the emission of a single molecule. This can be achieved by bringing a metallic tip (such as those used for scanning tunneling microscopy) so close to a molecule that its radiation toward a detector is increased (*6*). Metallic-tip nanoantennas of this kind have been used to study the fluorescence or Raman scattering of nanometer-scale objects (*7*–*9*).

The next step is to improve the design and the performance of the nanoantennas. A possible approach is to reproduce classical radio antenna designs at the nanometer scale. Two groups have recently succeeded in building such nanoantennas. Schuck *et al.* obtained a strong enhancement of the field with a nanometer-scale bow-tie antenna (*10*). Mühlschlegel *et al.* (*1*) now report an optical version of the simplest radio antenna: a half-wave antenna. This antenna consists of a wire with a length of half a wavelength at the operating frequency; as a result, the current can resonate along the wire with a maximum at its center and zero current at both ends. The authors observe a resonant enhancement of the radiated power as the antenna length is varied, a behavior similar to the length dependence of a half-wave radio antenna.

Unlike radio antennas, the optical antenna strongly depends on electron resonances in the metal of the antenna (*11*). Taking advantage of these resonances is another promising approach to modify spontaneous emission. Antennas based on the excitation of surface waves (*12*), or on guided waves (*13*) that are subsequently diffracted, may be used to produce highly directional emission (or, to put it in electrical engineering terms, a large gain). This type of structure can also be used to substantially enhance the power emitted by fluorophores (*14*). Given the applications for light sources and quantum information, there is little doubt that research into nanoantennas will continue to grow rapidly.

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IMMUNOLOGY

Opposites Attract in Differentiating T Cells

Mark Bix, Sunhwa Kim, Anjana Rao

During differentiation, precursor cells with progressively narrowed potential give rise to progeny cells that adopt one of two (or more) divergent cell fates. This choice is influenced by intricate regulatory networks acting at multiple levels (*1*). Early in differentiation, precursor cells show low-level activation of all progeny genetic programs. Bias toward a given lineage comes from environmental inputs that activate powerful positive- and negative-feedback loops, which work in concert to impose selective gene expression patterns. Two recent papers, by Hwang *et al*. in *Science* (*2*) and Spilianakis *et al*. in *Nature* (*3*), suggest unusual mechanisms that modulate T cell differentiation.

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Mammalian defenses against pathogens are regulated by recognition of specific cues—whether the pathogen is intracellular or extracellular, and whether it bears flagella or produces double-stranded RNA. Upon recognition of these signals together with appropriate antigen, "naïve" progenitor CD4 T cells differentiate into T helper 1 (T_H1) or T helper 2 (T_H2) effector cells, which are optimally suited for control of intracellular and extracellular pathogens, respectively. During T_H1 differentiation, the interferon-γ gene (*Ifnγ*) on mouse chromosome 10 is activated for high-level transcription, whereas the clustered interleukin-4 (*Il4*), *Il13*, and *Il5* genes on mouse chromosome 11 are silenced. The reciprocal pattern is observed in differentiating T_H2 cells. All these cytokines are produced at low levels during the first stages of antigen recognition by naïve T cells (*4*).

Differentiation toward the T_H1 and T_H2 fates is self-reinforcing (*4*). IL-4 is not only a T_H2 effector molecule, but is also a key developmental determinant. IL-4 produced by activated naïve CD4 T cells participates in a positive-feedback loop, activating STAT6. In turn, STAT6 up-regulates GATA3, a transcription factor that specifies many features of T_H2 fate. Similarly, production of IFN-γ by naïve CD4 T cells directs STAT1-dependent formation of an IL-12 receptor that transduces IL-12 signals generated by the microbial recognition system into STAT4-mediated activation of T-bet, a T-box transcription factor that specifies many aspects of T_H1 fate. GATA3 and T-bet autoactivate their own expression, thus stabilizing lineage commitment (*4*).

Like other antagonistic cell differentiation pathways, T_H1 and T_H2 differentiation are mutually exclusive, with positive regulators of one pathway typically acting as negative regulators of the opposing pathway (*1, 4*). Thus, IL-4 inhibits T_H1 differentiation, in part because GATA3 down-regulates the receptor for IL-12. Conversely, IFN-γ blocks T_H2 differentiation, in part by impairing signaling responses to IL-4. Glimcher and colleagues (*2*) now demonstrate a surprising direct interaction between the two opposing lineage-specific transcription factors GATA3 and T-bet (see the figure). They show that activation or T_H1 priming of naïve CD4 T cells causes rapid tyrosine phosphorylation of T-bet by the kinase Itk, a modification that is not observed upon activation of differenti-

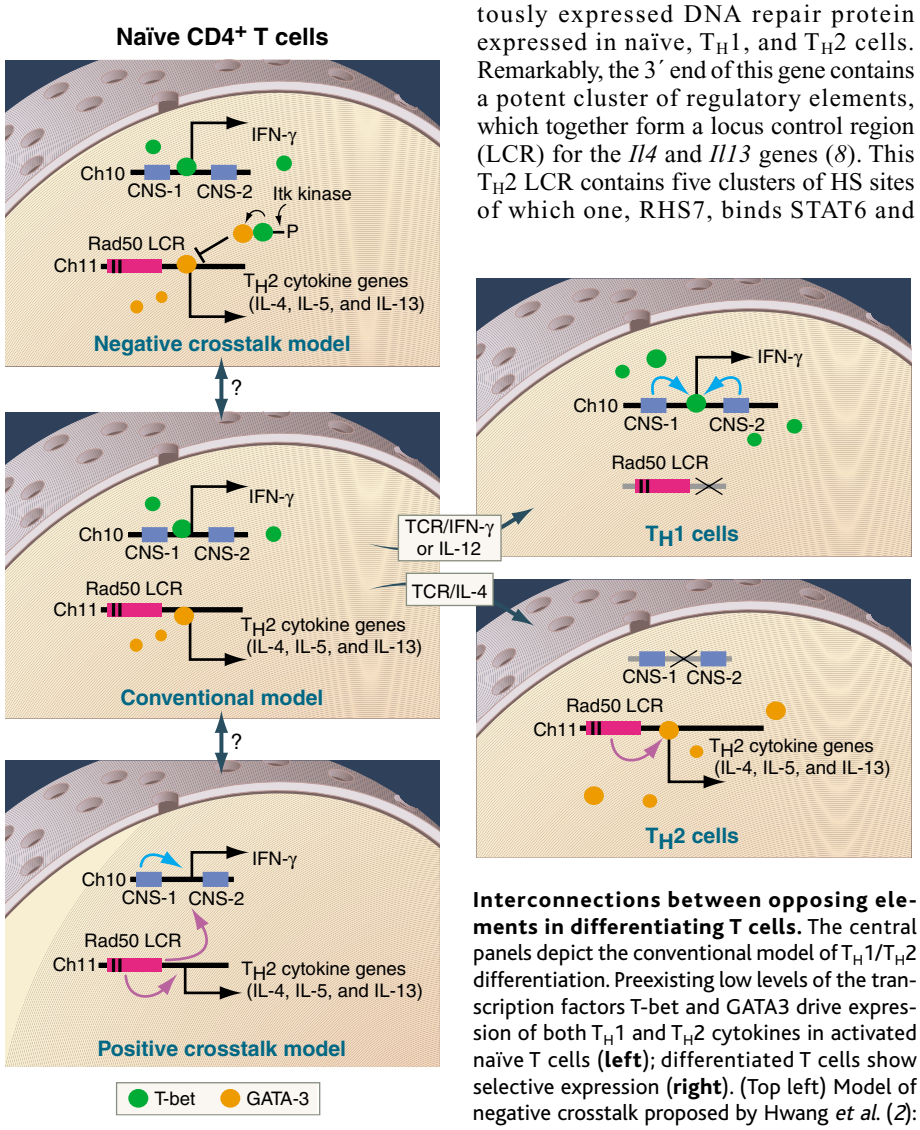
ated T_H1 cells. Tyrosine phosphorylation is not required for T-bet to activate IFN-γ. Instead, it promotes an interaction between T-bet and GATA3 that inhibits DNA binding of GATA3 (*3*). In this case, Itk promotes T_H1 differentiation. However, lack of Itk is also associated with a T_H1 bias—under conditions of low-avidity T cell receptor (TCR) stimulation $Itk^{-/-}$ T cells up-regulate T-bet and produce IFN-γ (*5*). In summary, Itk is required for optimal production of both T_H1 and T_H2 cytokines, but its contribution to lineage commitment varies depending on external signals and strength of TCR stimulation (*5*).

While signaling networks initiate T_H1 and T_H2 differentiation, chromatin-based mechanisms stabilize these fates. The mouse *Il4-Il13* locus contains 13 clusters of deoxyribonuclease I hypersensitive (HS) sites, most of which correspond to phylogenetically conserved noncoding sequences (CNSs) (*6*). These sites likely mark locations of cis-acting regulatory elements that transduce the outputs of signaling networks into transcriptional state changes (*6*). Targeted deletions encompassing T_H2-specific HS sites impair but do not abrogate T_H2-specific expression of *Il4*, *Il13*, and *Il5* (*4*, *6*). Conversely, targeted deletion of an HS site that is present in T_H1, T_H2, and naïve CD4 T cells impairs T_H1-specific silencing of *Il4* (*7*). Thus, T_H2 cytokine gene regulation is achieved by coordinated activation of multiple dispersed enhancer- and silencer-like elements.

Between the *Il5* and *Il13* genes in the T_H2 cytokine cluster (*Il5-Rad50-Il13-Il4*) is the *Rad50* gene, which encodes a ubiquitously expressed DNA repair protein expressed in naïve, T_H1, and T_H2 cells. Remarkably, the 3′ end of this gene contains a potent cluster of regulatory elements, which together form a locus control region (LCR) for the *Il4* and *Il13* genes (*8*). This T_H2 LCR contains five clusters of HS sites of which one, RHS7, binds STAT6 and functions as a T_H2-specific enhancer (*9*–*11*). Although less well characterized than the T_H2 cytokine locus, the *Ifnγ* locus also contains CNSs (CNS1-*Ifnγ* and CNS2-*Ifnγ*) that act as distal enhancers (*12*, *13*).

Recent studies of chromatin topology at the β-globin locus using the powerful new chromosome conformation capture (3C) assay have revealed intrachromosomal clustering of individual globin promoters with HS sites in the globin LCR, in a structure dubbed the "active chromatin hub" (*14*, *15*). At each developmental stage, the transcriptional activity of a globin promoter appears to correlate with its presence in the "hub." Application of the 3C assay to the *Il4-Il13-Rad50-Il5* locus reveals a similarly complex picture of intrachromosomal clustering. In fibroblasts that do not transcribe T_H2 cytokine genes, the *Il4*, *Il13*, and *Il5* promoters form a core interacting structure, whereas in naïve T cells that are equipped to transcribe the genes, the interactions are extended to include the T_H2 LCR (*16*). An attractive hypothesis is that the promoter-LCR interactions confer a "poised" conformation on the locus, which gives naïve T cells the ability to express T_H2 cytokine genes rapidly. Indeed, T cells with a targeted deletion of the T_H2-specific RHS7 HS site in the T_H2 LCR show diminished cytokine production as well as diminished T_H2 promoter-LCR interactions (*11*). Surprisingly, a very similar, although stronger, hub configuration is observed in differentiated T_H1 and T_H2 cells, implying that the current resolution of the 3C technique is insufficient to reveal subtle changes associated with gene activation and silencing.

To this evolving story, Flavell and co-workers add an interesting new wrinkle: interchromosomal interactions between the chromosome 10 *Ifnγ* locus and the chromosome 11 T_H2 cytokine locus (*3*). Using the 3C technology, they show that in naïve CD4 T cells, *Ifnγ* interacts with the promoters for *Il5* and *Rad50* as well as RHS6, a cluster of HS sites adjacent to RHS7 (*9*, *10*). All three interactions are stronger in naïve than in differentiated T cells, and appear to be specific because other sites across the T_H2 cytokine locus and several control regions on other chromosomes are not detected as part of the chromatin hub. The authors propose that interchromosomal interactions in naïve T cells are replaced during differentiation by more conventional intrachromosomal interactions involving cis-regulatory elements of the cytokine genes (see the figure). Notably, the *Il5* promoter (*Il5p*) is involved in interchromosomal (*Il5p-Ifnγ*) as well as intrachromosomal (*Il5p-Il4p-Il13p*) interactions in naïve T cells, suggesting either very dynamic interactions or the possibility of heterogeneity in the naïve progenitor population.



Interconnections between opposing elements in differentiating T cells. The central panels depict the conventional model of T_H1/T_H2 differentiation. Preexisting low levels of the transcription factors T-bet and GATA3 drive expression of both T_H1 and T_H2 cytokines in activated naïve T cells (**left**); differentiated T cells show selective expression (**right**). (Top left) Model of negative crosstalk proposed by Hwang *et al.* (*2*): Itk-mediated tyrosine phosphorylation of T-bet prevents GATA3 from binding to its sites in T_H2 cytokine genes. (Bottom left), model of positive crosstalk proposed by Spilianakis *et al.* (*3*): Naïve T cells display both intra- and interchromosomal interactions involving the T_H2 cytokine locus and the *Ifnγ* gene. The RHS6 and RHS7 elements are indicated by black bars on the pink Rad50 LCR. In differentiated T cells (right), interchromosomal interactions are abandoned in favor of extended intrachromosomal interactions with additional distal regulatory elements.

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To confirm and refine the 3C observations, the authors used DNA-FISH (fluorescence in situ hybridization). The observed interchromosomal interactions are largely monoallelic and occur in only a minority of cells (~30 to 40%). As with the 3C technique, the percentage of cells with colocalized alleles was higher in naïve than in differentiated cells, and no colocalization was detected in fibroblasts that do not express the cytokine genes. Because most colocalized genes are not located in heterochromatic regions identified by HP-1 staining, the authors propose that the genes are held in an environment that "poises" them for rapid expression upon stimulation. It would be interesting to test whether this nuclear compartment also holds other genes, such as *Il2*, known to be rapidly expressed by naïve T cells.

The hypothesis of a poised nuclear compartment is not fully borne out by data comparing wild-type and RHS7$^{-/-}$ T cells. The 3C technique gives the expected result: RHS7$^{-/-}$ cells showed decreased interaction of *Ifnγ* with the *Il5* promoter and RHS6 relative to wild-type cells. However, by DNA-FISH, more RHS7$^{-/-}$ than wild-type cells displayed colocalized *Ifnγ* and T_H2 cytokine loci (although colocalization was not as "close" in RHS7$^{-/-}$ as in wild-type cells). Consistent with decreased interaction between *Ifnγ* and the T_H2 LCR, RHS7 deletion resulted in slower *Ifnγ* transcription without affecting expression kinetics of *Il5* or *Rad50*, which are not controlled by the T_H2 LCR. The impairment in *Ifnγ* transcription in RHS7$^{-/-}$ cells could be a nonspecific outcome of introducing a deletion in the T_H2 LCR, or it could result indirectly from the known effects of RHS7 on *Il4*, *Il5*, and *Il13*. RHS7 might also have a cis influence on genes such as *Irf1* (also linked to the *Il4* locus) that would then affect *Ifnγ* expression indirectly. Additional studies will be needed to resolve these issues.

T_H1 and T_H2 cell differentiation are critical for proper immune responses, and imbalances in the function or activity of these cell types are responsible for many immune diseases, including autoimmunity and asthma. Understanding the mechanisms regulating the development and function of these cell types is therefore important from a clinical perspective. Equally interesting is the possibility of using the well-characterized program of T_H1/T_H2 lineage commitment to unravel the complex interconnections of regulatory networks that control cell differentiation.

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PSYCHOLOGY

Appearance DOES Matter

Leslie A. Zebrowitz and Joann M. Montepare

Take a look at these two snapshots (see the figure). Which man is more babyfaced? Most viewers would say it's the person on the right. And that's the person who lost a 2004 U.S. congressional election to his more mature-faced and competent-looking opponent. In fact, about 70% of recent U.S. Senate races were accurately predicted based on which candidates looked more competent from a quick glance at their faces. This remarkable effect, reported by Todorov *et al*. on page 1623 of this issue, likely reflects differences in "babyfacedness" (*1*). A more babyfaced individual is perceived as less competent than a more mature-faced, but equally attractive, peer of the same age and sex (*2*, *3*). Although we like to believe that we "don't judge a book by its cover," superficial appearance qualities such as babyfacedness profoundly affect human behavior in the blink of an eye (*4*).

What facial qualities make someone look more babyfaced and less competent? Facial measurements and computer modeling reveal that babies and babyfaced adults of all ages share such features as a round face, large eyes, small nose, high forehead, and small chin (*2*, *3*, *5*). So a babyish face is not synonymous with age, which Todorov *et al*. (*1*) eliminated as an explanation for their findings. This general quality also seems to be racially universal and evident in both sexes (*2*, *3*). However, a woman's facial anatomy tends to be more neotenous than a man's, which may be a disadvantage for women when vying for leadership positions (*6*). The association between facial maturity and perceived competence is ubiquitous: Babyfaced individuals within various demographic groups are perceived as less competent, whether by their own or another group. Its impact can be seen even for famous politicians: When images of former U.S. presidents Reagan and Kennedy were morphed to increase babyfacedness, their perceived dominance, strength, and cunning decreased significantly (*7*).

Which person is more babyfaced?

Why do we think babyfaced people are less competent, at first glimpse? According to the ecological theory of social perception, our ability to detect the attributes of age, health, identity, and emotion has evolutionary and social value. Thus, we have a strong, built-in, predisposition to respond to facial qualities that reveal these characteristics. Moreover, our responses can be overgeneralized to people who look like individuals who actually have the attributes. In this case, our impressions of babies (submissive, naïve, and weak) are extended to babyfaced adults who are consequently perceived as less competent than their more mature-faced peers. On the other hand, we get a more warm and honest impression from a babyface (*2*, *3*, *5*).

So what are the social—even political—consequences of our behavior? One must consider the context. Just as competent-looking, mature-faced individuals are favored as congressional leaders, so are

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CREDIT: FIG. 1A FROM TODOROV *ET AL.* (*1*)

they favored for other occupations requiring leadership and intellectual competence. However, those occupations requiring warmth, such as nursing, are most likely assumed by babyfaced adults (*2*, *3*). Contextual effects are also seen in judicial decisions. Judges are more apt to believe denials of negligent acts by mature-faced defendants, whose competent appearance is inconsistent with carelessness. In contrast, they believe denials of intentional transgressions by babyfaced defendants, whose warm and honest appearance is not compatible with such malfeasance (*2*, *3*). Shifts in the popularity of American actresses tell a similar tale regarding contextual relevance of perceived competence. Actresses with mature faces are favored during times of social and economic hardship. But in prosperous times, we turn our preference toward those with a baby's glow (*8*).

When does perceived competence fail to predict election outcomes? Todorov *et al*. found that more competent-looking candidates were defeated in 30% of races. One possible explanation is that face biases could have favored babyfaced candidates in those particular contests. It would be interesting to determine whether babyfaced candidates have the edge in races where polls show that integrity is a highly relevant trait. Like competence, perceived integrity is an important quality used to judge politicians, and it favors babyfaced individuals (*9*, *10*). The more competent-looking candidates also had only a small advantage in contests between candidates of different sexes. This was attributed to people's reluctance to judge the relative competence of male versus female opponents (*1*). Such concerns should be minimal when judging babyfacedness. Thus, we may better predict outcomes in mixed-sex contests if babyfacedness is used as a proxy for perceived competence.

Are we far from predicting the winner of an election based on voters' responses to a candidate's appearance? Unfortunately, the Todorov *et al*. study shows that this reality may be all too near. The study has important implications for political marketing, social decision-making, and the democratic process. It also highlights unanswered questions about appearance biases at both the neuroscience and social science levels. What brain mechanisms underlie automatic reactions to superficial qualities such as facial appearance? How can we inoculate people against biased reactions to such qualities? The latter question is particularly important given that more competent-looking victors in congressional elections are not likely to be smarter or bolder than babyfaced losers. Indeed, Todorov *et al*. noted that more babyfaced men tend to be slightly more intelligent (*1*). They also tend to be more highly educated, contrary to impressions of their naïveté, and more assertive and more likely to earn military awards, contrary to impressions of their submissiveness and weakness (*2*, *3*). Understanding the nature and origins of appearance biases has real-world value, not the least of which may be identifying electoral reforms that could increase the likelihood of electing the most qualified leaders rather than those who simply look the part.

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MATERIALS SCIENCE

Snapshots of Crystal Growth

Michael D. Ward

Crystallization is essential for the manufacture of products as varied as electronic devices, large-tonnage commodity materials, and high-value specialty chemicals such as pharmaceuticals. Yet our understanding of the crystallization process remains limited, especially for organic, polymeric, and protein crystals. Once a crystal has formed, its internal structure can be determined by x-ray diffraction, but unraveling the key steps in its crystallization requires tools that allow control and microscopic visualization of crystal growth, particularly at the early stages that often determine crystal properties such as defect density, purity, size, morphology, and polymorphism (the ability of a material to adopt different crystal structures).

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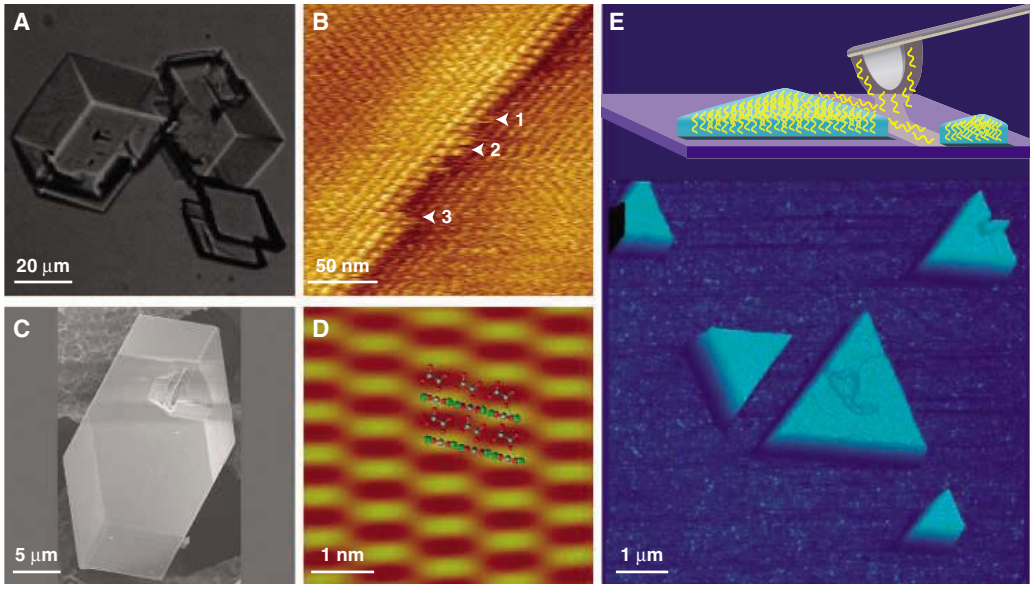
Several research groups have used atomic force microscopy (AFM) (*1*) to achieve a comprehensive understanding of crystal nucleation and growth at microscopic length scales, building on early studies that established the capability of this technique for examining crystal surfaces and crystallization (*2*–*5*). The use of AFM for crystallization studies involves scanning a small tip (often made from silicon or silicon nitride) over a crystal surface immersed in the crystallization medium. This approach reveals the two-dimensional lattice structure of the exposed crystal surfaces and permits direct visualization of surface features (such as terraces, ledges, and kinks) to which incoming molecules attach.

In situ AFM imaging of this kind is particularly well suited to small-molecule or protein crystals, which are inherently soft and easily damaged when studied in air or a vacuum. In liquid media, these materials are less susceptible to mechanical damage by the scanning tip, because the forces between the tip and the sample are smaller than they would be in air or vacuum (*6*). Moreover, a liquid medium precludes capillary condensation between the tip and the sample, which would otherwise create meniscus forces that can damage soft surfaces during imaging. Combined with the real-time imaging capability of AFM, the weaker tip-sample forces in liquids enable reliable real-time visualization of crystal growth in situ (*7*).

In a typical AFM study of crystal growth, a pregrown crystal is affixed to an AFM sample stage or is crystallized on a surface exposed in the AFM cell. Crystal growth is then prompted by a change in concentration or temperature. For example, the growth of crystalline organic conductors can be regulated precisely through adjusting the electrochemical potential, which governs the solute concentration at the crystal interfaces (*4*, *8*).

AFM has proven especially useful for examining the crystallization of proteins, including lysozyme (*2*), canavalin (*9*), hemoglobin (*10*), and insulin (*11*). Most insulin crystals (see the figure, A) consist



Imaging crystal growth with AFM. (**A**) Insulin crystals. (**B**) An AFM image of an insulin crystal surface. See text for description of arrows. (**C**) A single crystal of calcium oxalate monohydrate (COM). [Adapted from (*16*)] (**D**) A lattice image of a COM surface reveals the crystalline order. The overlay represents the molecular structure of the equivalent plane in the bulk crystal. [Adapted from (*16*)] (**E**) Poly-DL-lysine hydrobromide (PLH) crystals generated on a mica surface by dip-pen nanolithography. In this method, an AFM tip delivers PLH to the surface (top) while simultaneously recording crystal growth (bottom). [Adapted from (*20*)]

of layers of hexagonally close-packed hexamers, each ~5 nm in diameter, which can be distinguished by AFM. A stunning image of an insulin crystal surface during growth (see the figure, B), shows a discrete insulin hexamer (arrow 1) and a pair of hexamers (arrow 2) attaching to a well-defined step that separates two terraces on the crystal surface (*12*). The image also reveals a double kink (arrow 3) along the step. The observations provide support for the terrace-ledge-kink model of crystallization, in which individual growth units—in this case, the insulin hexamers—attach to steps (which separate terraces) to generate kink sites where other growth units subsequently attach. The ability to view crystallization events directly, at the level of the individual growth unit, promises insights into the influence of experimental condition on crystallization at the near-molecular level, rather than by inference from characterization of bulk crystals.

The crystallization of calcite, a ubiquitous biomineral, has also been studied extensively (*13*). Collectively, these studies have demonstrated the temporal evolution of crystal faces during growth as well as the influence of additives on growth. Particularly interesting is the appearance of chiral shapes on the crystal surface in the presence of D- or L-aspartic acid. This behavior signals symmetry breaking due to specific binding interactions between the chiral molecules and certain calcite crystal planes (*14*), which suggests a possible origin for the chiral morphologies of many biomineralized structures.

The application of AFM to biominerals has recently been extended to single crystals of calcium oxalate monohydrate (COM) (see the figure, C), the primary ingredient of most kidney stones. Near equilibrium, at which the crystal surfaces are neither growing nor dissolving rapidly, AFM reveals that the exposed surfaces of these crystals are highly ordered, with lattice structures essentially identical to those of the corresponding crystal planes in the bulk crystal (see the figure, D). The images in B and D illustrate the capability of AFM as a crystallographic tool that permits assignment of the crystal faces and their associated steps, which is critical for elucidating growth mechanisms.

AFM has been used to measure the effect on COM crystal growth of various additives, including citrate and osteopontin (*15*) (which regulate kidney stone formation in vivo) and carboxylate-rich synthetic polymers (*16*). These studies reveal that the binding of molecular species to crystal planes is highly specific. The fundamental understanding gleaned from these and related investigations (*17*, *18*) promises a path to the prevention of stone disease.

In a recent twist, dip-pen nanolithography (*19*) was used to deliver poly-DL-lysine hydrobromide (PLH) molecules from an AFM tip to a mica surface while simultaneously visualizing PLH crystal formation and growth in situ (*20*). The AFM tip served as a miniature pipette that delivered a high concentration of molecules to the surface, resulting in the formation of crystals with well-defined triangular habits in the area over which the tip was scanned (see the figure, E). Successive images revealed crystals growing continuously during scanning, concomitant with the generation of new crystals.

This approach enabled spatial control of crystal nucleation, and the crystal growth rate was regulated by adjusting the residence time of the AFM tip over the substrate. The triangular crystals adopted one of two orientations on the mica surface that differ by a 180° rotation, signifying epitaxy (an energetically preferred alignment of the PLH and mica lattices). Because dip-pen nanolithography can be configured for massively parallel operations, the real-time imaging capability of AFM may prove useful for identifying new crystal forms and optimizing crystallization conditions.

These examples illustrate the real-time in situ imaging capabilities of AFM for characterizing crystal growth at the near-molecular level. The ability to perform such measurements under actual crystallization conditions can reveal the critical factors that govern crystallization. Future developments in AFM should advance our understanding of crystal growth even further.

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INTRODUCTION

Vive la Différence

Many a song and poem celebrates the splendid differences between men and women. But obvious physical variation aside, in what ways do we differ mentally, physiologically, and socially? This special issue on Women's Health highlights many points of divergence. However, the title of this issue should not prompt the male reader to put down this copy and wait for a subsequent one. The issue covers many topics that concern men and women equally, such as the HIV/AIDS pandemic, cardiovascular disease, sexuality, and personalized medicine, as well as matters specific to women.

Much of our understanding of sex variation stems from the Women's Health Initiative, an effort launched in the late 1980s to probe gender questions and to increase the number of women in clinical trials. Without doubt, more women are now included in trials, and more studies focus on diseases that especially afflict women, but much improvement is needed; see the Editorial on p. 1517 and News story on p. 1570. Uterine fibroids, a major indication of hysterectomy, have received less attention. See Walker and Stewart on p. 1589. Likewise, mechanistic insight is needed to define preeclampsia, a circulatory disturbance between mother and fetus (see Redman and Sargent, p. 1592). In contrast, Berkley and colleagues (p. 1587) note that substantial progress has been made in understanding endometriosis, particularly the pain associated with it.

The understanding of male/female differences in disease manifestation and drug response has also lagged behind (see News story on p. 1572). For instance, investigators are beginning to probe the differences in brain chemistry and anatomy that contribute to the different patterns of mental illness in men and women (see News story on p. 1574). In a related story (p. 1576), Miller describes how HIV/AIDS and other factors seem to increase risks to mental health for women in developing countries. Greater awareness about gender variation also reveals an increasing "feminization" of the HIV/AIDS pandemic, due in part to the heightened social and biological vulnerability of women, as Quinn and Overbaugh describe on p. 1582.

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In dealing with topics that differentiate the sexes, the heart emerges as central. Mendelsohn and Karas (p. 1583) review how the molecular and cellular physiology of the heart and blood vessels differ between males and females during development and in cardiovascular disease. And a News story on p. 1580 reports on gender differences in bone quality and fracture risk, an issue of increasing importance to both sexes in an aging population. Finally, touching on a hot-button topic, Enserink (p. 1578) explores what some call the "medicalization" of female sexuality and whether, in the age of Viagra, lack of desire is a disease requiring drug therapy.

Related materials include Policy Forums in which Greenberger and Vogelstein (p. 1557) and Manasse (p. 1558) debate some pharmacists' refusal to dispense prescriptions for contraception, a Book Review by Aplin on pregnancy loss (p. 1555), and multiple online articles in the Signal Transduction and Science of Aging Knowledge Environments on topics such as cervical cancer and the role of estrogen in diseases of aging.

In all, this issue contains ample fodder for the ongoing debate about what men and women share and how they are unique—la différence.

–BEVERLY PURNELL, LESLIE ROBERTS, ORLA SMITH

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REVIEWS

See also the Editorial on p. 1517; the Book Review on p. 1555; the Policy Forums on pp. 1557 and 1558; SAGE KE material on p. 1511; STKE material on p. 1511; and www.sciencemag.org/sciext/womenshealth for related online material.

Science

NEWS

From Dearth to Deluge

The charge that women were being excluded from clinical studies led to the Women's Health Initiative; it produced a flood of data and controversy

At a congressional hearing 15 years ago, male leaders of the U.S. biomedical world endured a grilling about sex and science. Specifically, on 18 June 1990, Representative Henry Waxman (D–CA) asked the heads of the various National Institutes of Health (NIH) what they were doing to enforce a 3-year-old mandate to include women in clinical trials. (The NIH director's job was vacant at the time.) One by one, the witnesses acknowledged that they didn't have much to report. It was a pivotal moment, say advocates for women's health studies. It attracted the media and reduced an arcane debate about disease prevention and sex differences into a simple theme: Women were being excluded.

The drama had been carefully choreographed, recalls Phyllis Greenberger, executive director of the Society for Women's Health Research (SWHR) in Washington, D.C. In 1989, its leaders discovered that a government panel co-chaired by NIH official Ruth Kirschstein had found that "there was a dearth of women enrolled in clinical trials," as Kirschstein recalls. The panel had also instructed U.S. health agencies to insist that grantees recruit more women—or explain why they didn't intend to.

"That was the hook" on which to hang an indictment, says Greenberger: "Here was a mandate that said they were supposed to be doing something—and they weren't doing it." Meanwhile, SWHR's co-founder, NIH obstetrician-gynecologist Florence Haseltine, says that she used her own money to hire the lobbying team of Marie Bass and Joanne Howes to push the campaign, leading to a congressional audit and the 1990 hearing.

Although women were not systematically excluded from trials, later analysts have found (see sidebar) that they were kept out of some famous studies of heart disease in the 1980s. One of the male-only studies—with the acronym MR FIT—examined the benefits of a careful diet and exercise. Another, the Physicians' Health Study by Harvard Medical School in Boston, Massachusetts, found that middle-aged doctors who took a small dose of aspirin every other day had a significantly lower risk (44% less) of heart attack. For efficiency, it had enrolled 22,071 men (who were more likely than women to have heart attacks and yield data) but not one woman. Critics said the results were irrelevant for half the population.

Because of such criticism, NIH launched a companion aspirin study in the 1990s of 39,876 women. A decade later, in March 2005, this $30 million study reported that women were different after all: Unlike men, the aspirin takers did not have a significantly lower risk of heart attack, but they did have a somewhat lower risk of stroke.

The government also made some administrative changes in 1990, creating an NIH Office for Research on Women's Health, headed by Vivian Pinn, to see that sex differences were investigated. And in a move that surprised many, President George H. W. Bush chose Bernadine Healy—a cardiologist at the Cleveland Clinic in Ohio—to be the first woman director of NIH. Almost immediately, in 1991, Healy unveiled what she called "a moonwalk for women," the most ambitious trial undertaken by NIH, the Women's Health Initiative (WHI). Some warned that the project, designed to run for 15 years and recruit more than 160,000 women, would cost $1 billion. By official reckoning, however, it will only reach the $725 million mark in 2007.

From the start, WHI caught flak, Healy recalls. Critics said it wouldn't work because it was too complex and poorly designed; they also feared that not enough women would enroll. Healy battled "relentlessly" to get money for it, she says, adding that she helped get it entrenched by committing money to 40 study centers before she left NIH. She also says she helped fend off a move to end the trial in the Clinton Administration.

Getting results. The number of clinical studies on women's health has increased dramatically since the 1980s.

Now in its 13th year, WHI has been extended to at least until 2010 so that it can continue tracking women in a large observational study. In October, WHI plans to deliver findings from two key diet studies, on the effects of calcium supplements and vitamins. But by far the project's most dramatic moment came in July 2002, when it produced a stunning and unexpected result. Hormone replacement therapy, assumed to help avert heart disease and keep the brain healthy, actually elevated risks in older women.

Clinical bombshell

There was never any doubt, Kirschstein says: "WHI was Bernadine's baby." To make it work smoothly, Healy summoned the heads of 10 NIH institutes to weekly meetings. At the core of the project were three randomized clinical trials that recruited roughly 68,000 women aged 50 to 79. The studies were linked so that women could contribute data to more than one at once. One examined hormone use, and the other two were designed to test popular ideas: that a low-fat diet could reduce breast cancer, colon cancer, or heart disease; and that taking daily supplements of vitamin D and calcium could prevent osteoporotic bone breaks.

WHI also paid for a series of community projects designed to instruct women in

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NIH

NIH panel co-chaired by Kirschstein finds that too few women are in clinical trials, mandates more recruitment.

1987

Congressional hearing asks about women in clinical trials.

1990

ASSOCIATED PRESS

1991

Newly named NIH Director Bernadine Healy announces the Women's Health Initiative, a "moonwalk for women."

40 clinical centers set for funding under WHI.

WOMEN'S HEALTH INITIATIVE

1992

healthy living. Funded by NIH, they were carried out by the Centers for Disease Control and Prevention in Atlanta, Georgia.

WHI's largest component is an observational trial that enrolled 93,000 women and continues to collect blood, urine, and DNA; it will go for another 5 years, says Program Director Jacques Rossouw of the National Heart, Lung, and Blood Institute (NHLBI). Participants have given consent to future genetic studies, including possible commercial uses of the data. Healy says this part—modeled on a famous 50-year study of doctors in Framingham, Massachusetts—may be WHI's main legacy.

The best-known part of WHI, though, is the clinical trial of hormone supplements. It had been planned for years at NHLBI, says Rossouw, because of the booming use of female hormones. In 1990, Wyeth Pharmaceuticals of Collegeville, Pennsylvania, manufacturer of the most popular pill—containing conjugated equine estrogen with progestin—was seeking approval to market the drug as a heart disease preventive. "We would have done the trial anyway," says Rossouw, but "Healy was forceful, and the political climate was favorable; that was why she could get it funded so rapidly."

The "medical culture at the time," Healy recalls, was "basically to put every woman over the age of 50 on hormones until she stepped into the grave." She, too, believed hormones were beneficial but felt the evidence "wasn't sufficient." The trial had two arms. One gave placebo or therapy in the form of a pill containing estrogen and progestin—a hormone added to reduce estrogen's known risk of increasing uterine cancer. The other gave estrogen alone to women who had had hysterectomies.

The project had "many skeptics," Rossouw recalls. For example, some doubted that women would stay with the low-fat diet long enough to yield results. A review by the Institute of Medicine (IOM) in 1992 suggested that this part of the study should change its primary endpoint to look for heart disease benefits, not cancer reduction. The chance of failure was so high, the IOM group warned, that it would be "wrong" to invest so much money and "find after 14 years that little in the way of useful information had been learned." A member of that panel, epidemiologist Lynn Rosenberg of the Slone Epidemiology Unit at the Boston University School of Medicine, says today: "I will be very surprised if the results [of the diet trial] … show anything." The vitamin D–calcium trial, she thinks, is more likely to come up with significant results. Data from both are to be published in the fall.

One of the critics' biggest worries—as Rossouw recalls with irony—was that giving women a placebo would deprive them of the benefits of hormone therapy, possibly making the trial unethical. Indeed, the IOM panel predicted that WHI was "likely to terminate early because of evidence demonstrating [hormones'] protection against" coronary heart disease.

It did end early, but not because hormone therapy was beneficial: WHI officials reported in July 2002 that women who took the combination pill were more likely than those on placebo to develop invasive breast cancer—38 in 10,000 compared to 30 in 10,000. Risks for heart disease, stroke, and

Clinical Trials: Keeping Score on the Sexes

Biomedicine used to have a bias against including young women in clinical trials, says Ruth Kirschstein, a former director of the National Institutes of Health (NIH)—partly because of the thalidomide disaster. This drug, given to pregnant women to stop nausea in the 1950s and early 1960s, caused thousands of birth defects. After it was withdrawn, regulatory agencies directed that young women should be kept out of clinical trials to protect fetuses they might be carrying. That attitude lived on into the 1980s—long after new tests and testing methods had made it easy to identify early pregnancy and avoid risks, Kirschstein says. In 1987, a government panel she co-chaired found that U.S. agencies were slighting women's health and ordered that more women be included in research trials (see main text). Some trials, particularly big ones looking at heart disease, were designed to focus on male patients. Too often, and with too little evidence, results based on males were extrapolated to females, the review found.

Some independent analysts argue that, aside from the big heart studies, the imbalance was never that great. But since the initial complaints, both sides agree, NIH has tipped the balance to include more women. NIH's own evaluation of grants found in 1987 that only 13.5% looked at diseases unique to women. But then, only 6.5% were on diseases unique to men (*Science*, 11 August 1995, p. 766). In 1990, a General Accounting Office (GAO) study ordered by Congress found that NIH was being "very slow" in carrying out its own goals of recruiting more women and investigating physiological differences between the sexes. A sample of 50 grant submissions on diseases that affect both sexes, GAO found, included 20% that didn't mention the subjects' sex, and "some" that excluded women but didn't explain why. In a 2000 audit, GAO found that NIH had made "significant progress." Peer reviewers reported that 94% of grant proposals in 1997 met the standards for including women, and "more than 50% of the participants in clinical research studies that NIH funded" were women.

As it became clear that women were not being excluded from the new crop of trials, critics shifted ground. For example, in a paper released on 10 May, the Society for Women's Health Research (SWHR) in Washington, D.C., said that NIH is not doing enough to get researchers to run clinical trials in a way that will bring to light physiological differences between men and women. The report by Viviana Simon, Sherry Marts, and other members of the SWHR staff found that "a very small percentage" of all indexed NIH grants between 2000 and 2003 (about 3%) were awarded to study sex differences. It also found that the richest institutes—such as those dedicated to cancer, heart disease, and infectious diseases—scored low in the study's index of funding research on sex differences. Now that women are being included in trials, SWHR argues, researchers should be doing more to learn how they differ from men.

–E.M.

1993 Congress establishes the NIH Office for Research on Women's Health.

2002 WHI trial of combination hormone therapy stops because of cancer risks.

2004 WHI trial of estrogen-only therapy stops because of stroke risks.

2005 WHI low-fat diet trial and vitamin D–calcium trial due to report results.

2007 Cost of ongoing WHI projected to reach $725 million.

blood clotting were also higher, whereas risks for hip fracture or colon cancer were lower.

The announcement made front-page news and sent a shock through the more than 6 million U.S. women who were taking hormones. Many quit (*Science*, 19 July 2002, p. 325 and 1 November 2002, p. 942). Sales of the estrogen-progestin pill plummeted about 40% and never regained the lost ground.

Because cancer risks for estrogen alone were deemed much lower, this part of the WHI trial continued. In March 2004, a monitoring panel stopped it, too, because women on this therapy had a higher risk for blood clots and strokes than those on placebo. An analysis showed that estrogen gave no significant protection against heart disease. Later in 2004, an analysis of women over 65 in the estrogen-only group found that they had a somewhat elevated risk for dementia compared to those on placebo.

The adverse events were undeniable, but some experts criticized the way WHI officials and authors described and released the findings. Wulf Utian, for example, a reproductive endocrinologist at the Cleveland Clinic and executive director of the North American Menopause Society, charges that the government stressed negative results to "achieve maximal impact."

Rossouw acknowledges that the data were released in a dramatic way. He didn't give advance warning to drug companies, doctors, or professional societies, but he mailed the findings directly to study participants—to maintain "confidentiality," he says. And he held a press conference because "the goal was to change medical practice." WHI succeeded, Rossouw thinks: Before WHI, people were trying to "get all older women on hormones," and afterward, the aim was to "minimize exposure." That is a "180-degree turnaround in medicine," Rossouw says—and one "we can feel gratified about."

Critics object, however, that WHI's specific results were used to discredit all hormone therapy. Utian suggests that the heart disease findings from women in the WHI group (median age 63) and dementia findings (age 65 and older) might not apply to younger women. For those just approaching menopause around age 50, he says, the benefits of symptom relief from hormones may outweigh other risks.

Endocrinologist Judith Turgeon of the University of California, Davis, also points out that alternative formulations may be less risky than the hormone pills used in WHI, which contain equine estrogens that can adversely affect the liver (*Science*, 28 May 2004, p. 1269). She notes that some researchers are testing lower drug doses or transdermal rather than oral administration.

Although WHI is not ready to conduct a big study of younger women—mainly because it would cost too much, says Rossouw—it is looking into a few lingering questions. For example, the WHI program is supporting an imaging study of women in the estrogen-only therapy group to look for reduced calcification of the arteries, a sign that may indicate a lower risk for coronary heart disease. WHI experts are watching the private Kronos Longevity Research Institute in Phoenix, Arizona, which is enrolling 720 women younger than the WHI profile in a trial that aims to test low-dose estrogen therapy and administration of estradiol by skin patch in combination with oral progesterone. If such studies are encouraging, Rossouw thinks, the government might consider a larger trial.

Healy argues that WHI's payoff will be greater than the sum of its findings. It proved that "big, strategic trials" of this kind can work, she claims, and that the government should not shy away from them. Most important, she says, WHI "absolutely blew open" the topic of women's health, which had been "terribly neglected."

–ELIOT MARSHALL

NEWS

Gender in the Pharmacy: Does It Matter?

Studies of how women's and men's bodies process drugs have turned up mostly minor differences. But some drugs may be less or more effective in women or cause more side effects, and other variations may await discovery

In 1989, a 39-year-old woman blacked out while she and her husband were eating dinner. He rushed her to the Naval Medical Center in Bethesda, Maryland, where tests showed that her heart had a dangerous irregular rhythm that can lead to cardiac arrest. Doctors were puzzled: The woman was taking a popular antihistamine, Seldane, overdoses of which had caused abnormal heart rhythm, yet she was taking the recommended dose. The doctors consulted Louis Cantilena, a clinical pharmacologist at the hospital, who in turn called a colleague at the U.S. Food and Drug Administration (FDA). Looking through FDA's adverse events database, FDA staffers found two dozen cases of arrhythmias from Seldane, or terfenadine—the majority in women. It was one of the first red flags that researchers might have been missing sex differences in responses to drugs.

Combing through data on other medications, FDA and researchers realized that at least nine drugs could cause potentially fatal heart arrhythmias in women, especially when prescribed with certain antibiotics. By 2001, FDA had pulled four of these drugs off the market, including Seldane. "There's no way to know how many, but there were deaths," says Raymond Woosley, then a pharmacologist at Georgetown University Medical Center in Washington, D.C., who began studying the problem.

The drug withdrawals fueled an argument made by advocates for women's health: Sex

Selected Medications and Possible Sex Differences

DRUG CLASS	WOMEN COMPARED TO MEN	STRENGTH OF EVIDENCE	MECHANISM
Certain antibiotics, antihistamines, antiarrhythmics, antipsychotics	Higher risk for drug-induced arrhythmias	Strong	Longer QT interval in women; drugs block cardiac ion channels
Opioids	May respond better to kappa-receptor opiates with fewer side effects	Mixed	Estrogen's effects on receptor density, binding, signaling
Antidepressants	May respond better to selective seratonin reuptake inhibitors	Mixed	Estrogen may enhance seratoninic effects
Anticoagulants (warfarin, heparin)	Bleeding more common	Strong	Doses too high for body size, possible pharmacodynamic effects
Antipsychotics	Respond better but more side effects	Strong	Fat-soluble so remain in women's bodies longer; estradiol may act on same receptors
Verapamil (hypertension)	Blood levels higher for oral drug, lower for intravenous drug	Strong	Activity of metabolizing enzyme (CYP3A4) and P-glycoprotein drug transporter

differences in responses to drugs had been missed because women were not always included in clinical trials, or if they were, the data were not broken down by sex. That has changed considerably in the past dozen years, after the National Institutes of Health brought more women into clinical trials and FDA rescinded a 1977 rule that excluded women of childbearing age from early trials (see p. 1570)—with positive results, advocates say. Earlier this year, for example, researchers reported in the *New England Journal of Medicine* (*NEJM*) that aspirin—which protects men against heart attack but not stroke—has exactly the opposite effect in women.

Yet sex differences in drug responses remain controversial. Concerns center on two aspects: how quickly drugs are metabolized and absorbed, and how they affect the body once they're in the bloodstream. Although studies have found many differences in how women and men process drugs, these changes are less worrisome than expected. Differences in how safe and effective a given blood level of a drug is for a man or woman are probably bigger issues, many experts agree. This is harder to study, however, and so far only a few clear-cut examples have emerged. That leaves some experts skeptical that sex will matter much in the long run; genetic variation among individuals, especially of different ethnicity, may dominate, they say. "Gender is not the major concern that we thought it would be," says Leslie Benet, a pharmacologist at the University of California, San Francisco (UCSF).

But others counter that drug researchers have barely scratched the surface. Despite prodding, clinicians still don't always analyze data on women separately, and more research—and better research tools—may yet reveal more serious gender differences, they say. Even subtle sex differences may be important in an era of personalized medicine. "When it's all done, we still find sex is a factor that keeps coming out," says clinical pharmacologist and cardiologist Janice Schwartz of UCSF.

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Less than expected

As far back as 1932, researchers noticed that female rats could be knocked out with half the dose of barbiturates needed for male rats of the same size. But such differences were largely ignored until 1993, when FDA reversed course on including women in trials. Until then, trial results were dominated by "the cult of the typical 70-kilogram male," says Sherry Marts, vice president of scientific affairs for the Society for Women's Health Research in Washington, D.C.

That began to change, slowly. The 1993 FDA guideline explicitly urged drug companies to look for sex differences in drug processing, or pharmacokinetics. Researchers examined old animal data and new human evidence suggesting that males and females differ in the activity of liver enzymes that metabolize drugs, particularly the cytochrome P450 enzymes, whose expression is modulated by sex hormones. One such enzyme, CYP3A4, is involved in metabolizing more than half of all therapeutic drugs; women clear some CYP3A4 enzyme drugs more quickly than men do and thus may need a higher dose to get the same effect. (The difference probably also involves women's lower liver levels of a protein called P-glycoprotein that shunts the drug out of cells in which CYP3A4 processes it.)

Women are also smaller on average than men are, they may absorb drugs more slowly, and their kidneys filter excreted drugs out more slowly. Because women tend to have more body fat, fat-soluble drugs stay in their bodies longer. All this means a woman who swallows the same number of pills as a man may end up with a larger or smaller level in her blood.

In many trials, however, differences in women's responses to a drug disappear if the dose data are simply adjusted for body weight or surface area. The gender differences in processing drugs that remain appear to be relatively minor, says pharmacologist Bernd Meibohm of the University of Tennessee, Memphis. The best evidence is an FDA study of the 300 new drugs reviewed from 1995 to 2000, more than half of which provided sex data. Only for 11 drugs were pharmacokinetic differences greater than 40%, and none resulted in separate dosing instructions for women—indicating the difference wasn't important to the clinical outcome, notes Margaret Miller, science program manager for FDA's Office of Women's Health. Pharmacologist Gail Anderson of the University of Washington, Seattle, is not convinced, pointing out that in negotiating labels with FDA, companies would resist dosing for subpopulations—or even for body weight—because it makes it harder to market the drug.

Metabolism differences in women do matter for drugs that must be given in very precise doses, such as the blood thinner warfarin and cancer drugs and immunosuppressive drugs. But doctors already carefully tailor doses of those drugs to the individual, notes Benet. "The bottom line is, [sex differences in pharmacokinetics] doesn't seem to make a major difference," agrees Meibohm.

The female heart

A potentially much bigger problem is the difference in how men's and women's bodies react to the drug once it's reached the bloodstream. Known as pharmacodynamics, this property of drugs is harder to measure: Gauging improvement in depression, for example, is trickier than detecting the blood level of a chemical. But disparities for some classes of drugs have emerged.

Probably best-established are differences in responses to medications that can affect the heart's rhythm, such as terfenadine. These include some antihistamines, antibiotics, antiarrhythmics, and antipsychotics. Woosley and others showed that these drugs share the ability to block potassium channels in the heart, which in turn can affect the heart's rhythm. Two-thirds of reported arrhythmias from these drugs occur in women; they are especially vulnerable because the female heart has a longer "QT interval," the time it takes to recharge between beats. More than 30 marketed drugs are known to cause arrhythmias, notes Woosley, president of the C-Path Institute in Tucson, Arizona. The University of Arizona lists these drugs on a Web site (www.qtdrugs.org).

Women also appear to respond differently to drugs for treating or preventing cardiovascular disease. The latest example is the study of aspirin reported in the 31 March issue of *NEJM* of results from the Women's Health Study, which was launched in 1993 after protests that a previous study had included only men. Whereas men were protected from heart attacks but not stroke, women 45 years or older who took low-dose aspirin for 10 years had no fewer heart attacks but a 17% lower rate of stroke. The results may involve differences in physiology in women, such as smaller coronary arteries than men have and lower lipid levels before menopause.

Sex differences also seem to come into play in the reaction to opiates. The strongest evidence comes from a series of studies in the mid-1990s led by Jon Levine's group at UCSF, which looked at how men and women respond to drugs known as kappa-receptor opiates after wisdom tooth surgery. The drugs worked much better on women and caused fewer side effects than opiates such as morphine that have a different target, mu receptors. Male and female rodents also respond differently to opiates.

But other groups haven't yet replicated the UCSF results, and in a controlled lab setting—for example, with volunteers subjected to mild heat and muscle pain—the same sex differences aren't always observed, cautions pain researcher Roger Fillingim of the University of Florida, Gainesville. The discordant studies may reflect factors such as the type of pain or opiate drug dose, Fillingim says. "I just don't think we have enough data" to know which conditions result in sex differences, he says.

The jury is still out on antidepressants as well. A fairly large study led by psychiatrist Susan Kornstein of Virginia Commonwealth University in Richmond and published in 2000 in the *American Journal of Psychiatry* reported that women responded better to selective serotonin reuptake inhibitors (SSRIs); men got more help from tricyclics, which target receptors for serotonin and other neurotransmitters. Not all subsequent studies have found these differences, however.

Under the radar

Many agree that important sex differences are yet to be discovered. For example, researchers noticed only in 2002 that an old drug for heart failure, digoxin, raised the death rate in women by 4% in an earlier trial, possibly because they received too high a dose, notes Schwartz. The painkillers known as COX-2 inhibitors, one of which was withdrawn from the market last year because of side effects, are also under the microscope. Concerns were raised after Garrett FitzGerald's group at the University of Pennsylvania in Philadelphia found that blocking the COX-2 enzyme in mice also hinders estrogen's protective effects against cardiovascular disease, suggesting that giving COX-2 drugs to young women could put them at higher risk for heart attacks and stroke (*Science*, 19 November 2004, p. 1277).

Pinning down sex differences should become easier with new biomarkers—such as brain imaging—that enable researchers to measure disease and other endpoints, such as pain, more objectively. "My guess is we're going to find a lot more gender differences," Woosley says.

Sex differences are also coming up in the context of pharmacogenomics: genetic differences, often tied to a single polymorphism, or mutation, that affect an individual's disease susceptibility, say, to heart disease or response to a drug. Although these mutations are usually not carried on the X chromosome and so are independent of sex, they can be modulated by sex hormones. For example, researchers recently found a polymorphism that makes redheaded women—but not men—more responsive to opiates, notes Fillingim, a co-author (*Science*, 16 July 2004, p. 328).

Even if most sex differences in drug responses aren't dramatic, they will feed into the cost-benefit tradeoff for a drug—all part of the personalized medicine equation, says Miller of FDA. "The most important message is to look for differences in treatment response by gender," says Kornstein, editor-in-chief of the *Journal of Women's Health*. She and others say their colleagues in drug research are listening, but not everyone is on the same page yet.

–JOCELYN KAISER

NEWS

Sex and the Suffering Brain

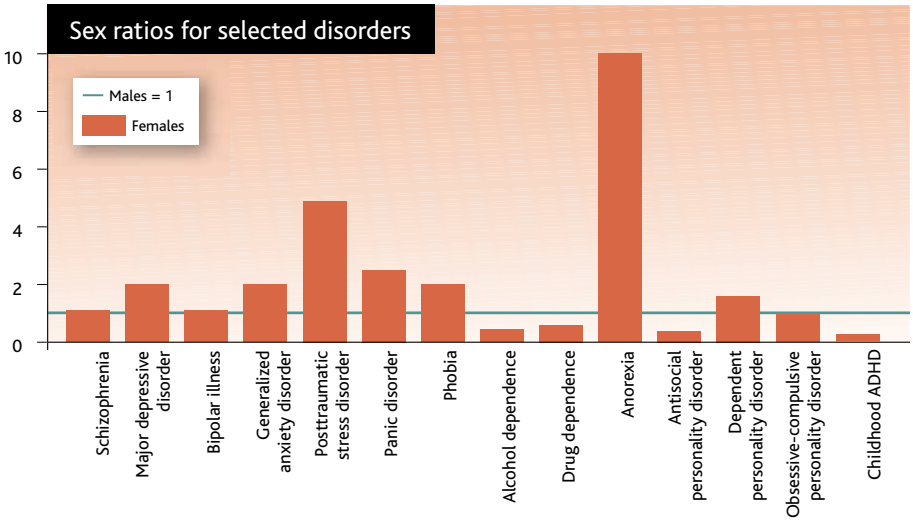
Researchers are seeking biological reasons for the widespread gender differences in the prevalence and symptomatology of mental disorders

It's easy to start a fight about whether there are gender differences when it comes to mental skills, but there's little debate that patterns of mental illness and disorders vary between the sexes. Women, for example, are more likely to get depressed (see table). Men are more severely afflicted by schizophrenia. Females have more anxiety. Males exhibit more antisocial behavior. Most alcoholics and drug addicts are male; females have more eating disorders. Even suicide has a gender bias. Females make more attempts; males are more successful.

Although culture helps shape how the two sexes express mental problems, some differences persist across cultures and across time, says psychiatrist Kenneth Kendler of Virginia Commonwealth University in Richmond. And that suggests a role for biology. In fact, says Thomas Insel, head of the National Institute of Mental Health (NIMH) in Bethesda, Maryland, "it's pretty difficult to find any single factor that's more predictive for some of these disorders than gender."

Talking about sex differences has long been taboo in some quarters—"People hear 'sex differences' and think you're talking about individuals, not populations," says Insel. "It's critical to remember there's a huge amount of variation within a population and overlap between populations." But neuroscience research, especially the explosion in brain imaging, has produced data that are hard to ignore. "Every time you do a functional MRI on any test, different parts of the brain light up in men and women," says Florence Haseltine, a reproductive endocrinologist at the National Institute of Child Health and Human Development (NICHD) in Bethesda, Maryland. "It's clear there are big differences." Understanding them will have "tremendous implications" for treatments of brain diseases and injuries, says Viviana Simon, director of scientific programs for the Society for Women's Health Research in Washington, D.C.

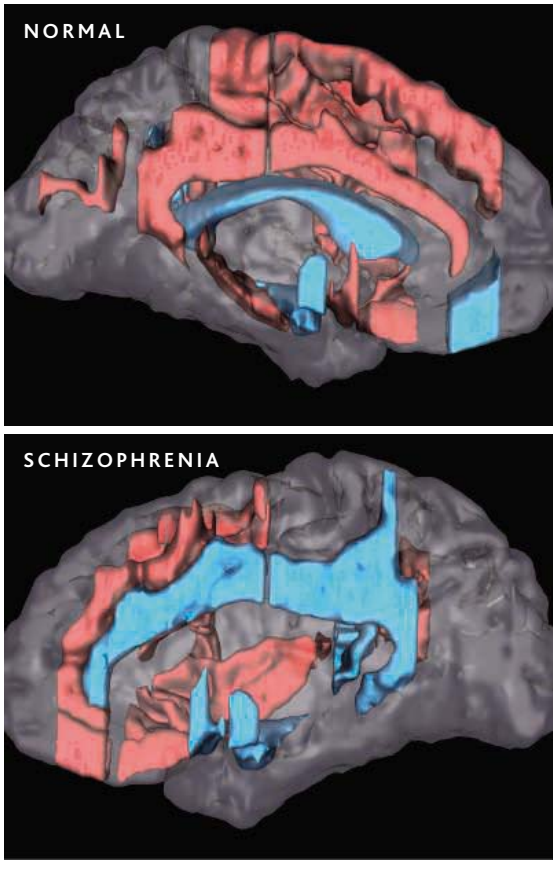
Most mental disorders are complex and resist the hunt for specific genes, yet family



SOURCE: NIAAA; NIMH; R. KESSLER/HARVARD UNIVERSITY

and twin studies have demonstrated significant heritability for them. These disorders interact with brain differences between the sexes that arise from genes on the X and Y chromosomes and from the bath of gonadal hormones that soak fetal brains early in gestation. Sex hormones are far-reaching in their powers, notes Insel. "They are sort of master transcription regulators; they affect hundreds of downstream genes. … There's no question these are big players in mental disorders." Those sex-related changes are sort of early filters, influencing the expression of underlying disorders in different ways, says psychologist Elizabeth Susman of Pennsylvania State University, University Park.

No one has managed to draw an unbroken line from prenatal development to adult behavior. But some researchers are now trying to tease apart just what aspects of brain anatomy and chemistry can help account for the gender skewing in mental disorders. "We're just at the beginning of trying to examine these differences," says Cornell University endocrinologist Margaret Altemus. Some studies are contradictory, and there is still more known about animals than about humans.



Schizophrenia switch. Certain brain areas are normally sexually dimorphic; different patterns occur on both sides of the brain in people with schizophrenia. The areas that are proportionately larger in females are shown in red; those for males in blue.

CREDIT: GOLDSTEIN *ET AL.*, *CEREBRAL CORTEX* (2001) AND *ARCH. GEN. PSYCHOL.* (2002)

Affective disorders

Epidemiologic studies show that women are more vulnerable than are men to most disorders that affect the emotions. These include major depression and a host of anxiety-related conditions, such as generalized anxiety disorder, panic disorder, posttraumatic stress disorder, and phobias.

Anxiety and depression are very closely related: Eye-blink tests reveal that a strong "startle response" is a good predictor for both. Negative experiences can trigger both anxiety and depression in vulnerable people. Those feelings involve the activation of multiple neurotransmitter and hormonal systems, including stress mechanisms that are heavily influenced by sex hormones.

The human stress response basically has two components: the autonomic nervous system that causes raised heartbeat, sweaty hands, and gut churning; and the slower-responding hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis involves a cascade of hormonal events that are normally counteracted by release of the stress hormone cortisol.

Because female depression rates start to rise during puberty, notes psychologist Laura Stroud of Brown University in Providence, Rhode Island, researchers posit that hormones play a role in women's vulnerability to affective disorders. Animal and human research has shown that sex hormones affect stress responses in different ways. Just the right amount of estrogen is required for emotional balance, says NICHD endocrinologist George Chrousos. Some women go into "withdrawal" and hence depression when levels drop. But too much estrogen can overactivate the HPA axis, also resulting in depression, he says. Testosterone, on the other hand, may protect against stress and depression through its damping effect on HPA reactivity. This is illustrated by a study appearing last month in *Neuropsychopharmacology*. Psychiatrist David Rubinow of NIMH and colleagues suppressed sex hormone production in 10 men and stimulated their HPA axes with corticotropin-releasing hormone (CRH). They found that testosterone replacement "significantly blunted" the cortisol response to CRH.

Other research shows that fear, a powerful stressor, may activate stress responses more in females than in males. Data from rats and mice "overwhelmingly indicate that females show more intense fear responses than males," says Jaak Panksepp of the Medical College of Ohio in Toledo. Testosterone appears to reduce males' reactions to pain, he adds. Human evidence is beginning to accumulate. In a study in the journal *Emotion* in 2001, researchers at the University of Florida (UF) observed the reactions of 50 women and 45 men to pictures of distressing things, such as car crashes and mutilations. They found that women had more extreme autonomic reactions as gauged by heart rate, skin conductance, and the startle response. "Women are more reactive on average in everything involving negative stimuli," says UF psychologist Margaret Bradley.

Brain-imaging studies are supplying a wealth of new data. A group at Westmead Hospital in New South Wales, Australia, recently completed a study (in press at *Neuroimage*) showing that females exhibited more widespread activation of the amygdala, the seat of the fear response—corresponding with rapid heart rate and sweating—than males did in reaction to pictures of people with fearful facial express-ions. In another study, in press at *Neuro-report*, a team headed by David Silbersweig of Cornell University's Weill Medical College in New York City found that normal females show more reaction to stress—in this case, anticipating a pain on the wrist—in the subgenual prefrontal cortex, a key region linked with anxiety and depression.

Although the evidence is more ambiguous, gender differences have also been seen in the HPA axis, the other component of the stress machinery. Observing 50 young volunteers, half of them women, Brown's Stroud found that in an "achievement" test, in which subjects must make a speech and perform a subtraction task in front of a panel of judges, men secreted higher levels of cortisol. But in a "social rejection" test, in which trained confederates made the subjects feel excluded in brief interactions, women's cortisol levels were higher, her team reported in the journal *Biological Psychiatry* in 2002.

NICHD's Chrousos contends that the HPA axis is slightly more reactive in females, as shown in a study in which young women responded with significantly higher levels of cortisol to a CRH challenge. Chrousos speculates that a highly tuned HPA axis, clearly evident in rodents and primates, is an evolutionary adaptation to help mothers protect their young.

Aggression and impulsivity

Sex hormones are also implicated in aggression-related gender differences, notes Kendler. There is abundant evidence, he says,

Poor Countries, Added Perils for Women

When the Indian government disbursed the first round of financial aid to families in Tamil Nadu state, hard hit by the 26 December 2004 tsunami, they doled it out to men, the traditional household heads. That didn't work too well, says K. Sekar, a psychiatrist with India's National Institute of Mental Health and Neurosciences, who has coordinated mental health support for the tsunami survivors.

Many men have coped with the disaster by drinking, Sekar explains, and much of the money intended for families flowed straight into state-run liquor stores. The second round of aid, delivered to women, seems to be doing more good, he says. Even though women—especially those who lost children in the tsunami—appear to have suffered most in psychological terms, they've handled it differently. Drinking is socially unacceptable for women, Sekar says, and they have largely internalized their distress, showing signs of anxiety and depression.

The psychological aftermath of the tsunami in Tamil Nadu reflects two of the most robust trends in psychiatric epidemiology: Across the globe, anxiety disorders and depression are more common in women, and substance abuse is more common in men (see main text). The situation there also hints at how social factors and women's roles as childbearers influence the mental health of women in developing countries, often for the worse.

Although there are no reliable figures on the prevalence of mental disorders for many parts of the world, there are signs that women in poor countries are more vulnerable than women in richer parts of the world, says Ricardo Araya, a Chilean-trained psychiatrist at the University of Bristol, U.K. A recent study by Araya and colleagues is the first to attempt a direct comparison of the gender gap in the prevalence of depression and anxiety disorders between developing and developed countries. The researchers interviewed more than 10,000 men and women in urban areas of Chile and the United Kingdom about their mental health and reported in the April issue of *Social Science and Medicine* that the gender gap is greater in Chile.

For women in sub-Saharan Africa, especially, HIV is a major risk factor for depression, says Sylvia Kaaya, a psychiatrist at Muhimbili University College of Health Sciences in Dar es Salaam, Tanzania. Although little research has been done to examine HIV's toll on women's mental health

Trying to cope. Many factors threaten the mental health of women in developing countries. The Asian tsunami, for instance, took a heavy toll.

CREDIT: AFP/GETTY IMAGES

that men are more prone to expressing unhappiness through an "externalizing pathway" of physical behavior that includes drinking, drug abuse, and violence, whereas women are more likely to "internalize," leading to depression and disorders such as anorexia.

The pattern of male externalizing, like sex differences in general, becomes more pronounced during puberty, when the hormones are flowing. "We know, from primate studies, that testosterone is directly related to aggression," says Kendler. "If you give females testosterone, they get more aggressive."

Addictions follow the pattern of male externalizing. Epidemiological studies have shown, for example, that in families of women with bulimia, the men often have alcoholism and other addictions. Studies have repeatedly shown that even within the alcoholic population, females are more often diagnosed with depression, whereas more males express antisocial behavior.

The sex-based tendency to act out versus internalize is evident in the distribution of the personality disorders, which involve maladaptive patterns of thinking and relating to the world. Some of these, such as dependent or histrionic personality disorders, are hotly debated, and critics argue that they only exemplify learned gender-typical behaviors. "Can it be that human beings manifest certain symptoms in ways that are politically and socially acceptable within certain historical times?" asks feminist therapist Arlene Istar Lev of Albany, New York. But psychiatrist Larry Siever of Mount Sinai School of Medicine in New York City says there's more to it. "It used to be a non sequitur to link biology and personality," he says. "Now we see a very real substrate."

Take borderline personality disorder (BPD), which features extreme emotional instability, impulsivity, and self-harming behavior and is most often seen in post-adolescent girls. Although people with BPD frequently have a history of childhood abuse, says Siever, brain scans of patients also show abnormalities. In an unusual study now under review at *Biological Psychiatry*, Siever and psychiatrist Antonia New also found sex differences. They compared the brains of 17 males and 9 females with BPD and a history of impulsive aggression to normal controls matched for sex. They report that the males with BPD showed less neural activity in prefrontal areas involved in inhibition. This "presumably suggests a brain mechanism" for this type of aggression, Siever says. The fact that males with BPD are more prone than women with BPD to impulsivity and aggression could partly explain why more women get the diagnosis, he adds; the men may be seen as having antisocial personality disorder (ASP).

Researchers are also looking into the biological dimensions of ASP, in which

in particular, Kaaya suspects that women experience extra stress that ups their risk of depression. Women have little say in negotiating condom use and other protective measures and are generally expected to care for infected relatives, Kaaya says.

Much of the research on women's mental health overall in developing countries has investigated its links to reproductive health. Women in poorer countries are more likely to have miscarriages or lose young children, and such events, especially when they occur more than once, take a heavy toll on a woman's psychological well-being, says Veena Das, an anthropologist at Johns Hopkins University in Baltimore, Maryland. Das has just completed a study in poor communities in Delhi, India, that documents sharply elevated rates of depression in women who have lost multiple pregnancies.

Women who give birth to healthy babies aren't immune either. Rates of postnatal depression run high in some developing countries. In India and Pakistan, for example, a handful of studies in the past few years have found that 20% to 30% of women suffer postnatal depression, about twice the prevalence in wealthy countries. That's not just bad for moms. A study published last September in the *Archives of General Psychiatry* found that Pakistani infants born to depressed moms were four times more likely to be underweight 6 months after birth.

Socioeconomic factors work against women in many societies, says Jill Astbury, a psychologist at Victoria University in Melbourne, Australia. Even in developed countries, she says, the most disadvantaged women—for example, single moms with low incomes, insecure work, inadequate housing, and lack of child care—have rates of depression two to three times higher than those of women in more favorable circumstances.

Unemployment and low income, aside from being bad for mental health in their own right, have been linked to high rates of another risk factor: domestic violence. "Factors in many developing countries such as low levels of education for women ... [and a] lack of legal redress and property rights in divorce make it more likely that women living with violent partners will be forced to stay with them to survive economically," Astbury says.

Such factors might help explain why some of the highest suicide rates in the world are found among women in developing countries. More than half of all female suicides worldwide take place in China, one of the few countries where more women than men die by suicide. A paper published last year in *The Lancet* reported a startling suicide rate of 148 per 100,000 among young women in Vellore, an inland city in Tamil Nadu, India. In the United States, roughly 4 women per 100,000 commit suicide each year.

Rising economies don't necessarily relieve the risk factors for poor mental health, however. A 1999 paper published in *Social Science and Medicine* suggested that growing income disparities created by rapid economic development in India, Chile, Brazil, and Zimbabwe may have increased the risk of anxiety and depression for women there. Development can add to the stress of everyday life for women, says Araya, one of the study's authors. As jobs become available, women are often expected to work outside the home in addition to their household duties. "They go and work in a sweatshop, and then they have to go home and cook," he says.

A more comprehensive view of women's mental health around the world should come from a massive international survey now under way in 28 countries. Ronald Kessler, an epidemiologist at Harvard Medical School in Boston who is directing the project for the World Health Organization, says it will examine a variety of potential influences on women's mental health, such as access to birth control, property rights, education, and reproductive history, including age of puberty.

Some researchers have proposed that sex hormones are responsible for the higher incidence of depression and anxiety in women, but the main evidence for that hypothesis is that these disorders appear in midpuberty in the United States, Kessler says. In developing countries, puberty is often delayed by several years as a result of malnutrition, even though girls marry and are thrust into adult roles earlier. Says Kessler: "This creates a natural experiment to tease out the relative effects of biology and social roles on female mental illness."

–GREG MILLER

males outnumber females three to one. People with ASP (formerly known as psychopaths) don't form deep attachments and feel little guilt. Psychologist Adrian Raine of the University of Southern California in Los Angeles believes he has hit upon a possible biological marker. He reported in 1997 in the journal *Child and Adolescent Psychiatry*, from a longitudinal study of 1800 children in the Republic of Mauritius, a strong correlation between slow heart rate among 3-year-old boys—suspected to reflect reduced autonomic reactivity—and their subsequent antisocial behavior as adolescents. A high threshold for reacting to physical or social threats can make for "fearlessness," which in turn inhibits the learning of normal social inhibitions, says Raine.

Susman of Penn State has similar findings. In a 1997 study she reported that adolescents who had low cortisol levels prior to an anticipated physical stressor exhibited raised antisocial behavior 1 year later. She believes these individuals are unable to "anticipate" stresses. "They're not good at planning or regulating," she says, so "they don't anticipate fear." It may be that males are more prone to hypoarousal of the stress response system and females to hyperarousal, speculates Susman.

Thought disorder

Sex differences also extend to cognitive functions such as memory, attention, and perception. Men's brains are more lateralized, which means that higher cortical functions tend to be centered in the right or left side of the brain, says genetic epidemiologist Kathleen Merikangas of NIMH, whereas in females there's more "crosstalk" between the sides and therefore more redundancy. Evidence for this comes from the fact that women are more likely than men are to recover language from strokes in the left hemisphere, where language is centered.

This redundancy may also be protective in girls, who have much lower rates than do boys of childhood developmental and mental disorders, including attention deficit hyperactivity disorder (ADHD) and autism, says psychiatrist Raquel Gur of the University of Pennsylvania in Philadelphia. Estrogen, too, says Gur, appears to have neuroprotective effects according to results of research on brain injury, epilepsy, and cognitive decline in aging.

Gur says these differences also seem to work against men afflicted with schizophrenia, the most complex and devastating mental illness of all. More males than females have schizophrenia, and they have earlier, more severe symptoms, says psychiatrist Jill Goldstein of Harvard University. In brain scans comparing men and women with schizophrenia, Goldstein has found that men tend to have greater deficits than women do in attention, language, visual-spatial perception, and other areas ruled by the cortex, such as olfaction and motor skills. These are also areas that she and others have found to be sexually dimorphic in normal subjects. She believes these deficits all begin prenatally during the period of sexual differentiation of the brain.

Researchers are still cautious about their conclusions. Despite evidence of a "huge number of ... differences between men and women's brains," says Cornell's Altemus, "it's hard to know which are functionally relevant." Nonetheless, times have changed, observes Goldstein: "For many years we were not even allowed to say there were sex differences in the brain."

–CONSTANCE HOLDEN

NEWS

Let's Talk About Sex—and Drugs

Seven years after Viagra was launched, many drugs are on the horizon to treat women's sexual problems. But several questions remain: Are they safe? And are they needed?

Laura was miserable. She had once enjoyed a healthy sex life, but any desire for sexual activity had vanished after her hysterectomy. Her 9-year marriage was on the rocks, and her husband was becoming emotionally abusive.

Her real-life story, told by marriage and sex therapist Jean Koehler to a panel of experts gathered by the U.S. Food and Drug Administration (FDA) in December, was meant to help persuade the committee to approve the first drug developed to treat women's sexual problems. Koehler had traveled from Louisville, Kentucky, to suburban Maryland to deliver 3 minutes of testimony on behalf of a testosterone patch called Intrinsa. Once her client started taking testosterone, Koehler said, her life changed for the better: She enjoyed sex again, her relationship improved, "and two little children were spared the trauma of impending divorce."

Not tonight. Waning desire is the most important sexual problem in women, clinicians say.

Intrinsa, developed by Procter and Gamble (P&G), is part of a wave of new drugs stirring controversy before a single one has hit the market. Advocates for these drugs, including some prominent researchers of women's sexuality, say they have the potential to help tens of millions of suffering women; not just those who have had their ovaries removed, which often happens as part of a hysterectomy, but "naturally menopausal" women as well. Even if the drugs don't promise to save a marriage, market analysts see them as potential blockbusters.

But the FDA panel was not convinced. At the end of its daylong session, the group unanimously rejected the drug. Panelists decided that there weren't enough data to show that long-term hormone treatment—patients have to wear the patch continuously—does not cause serious side effects. The decision, decried as overly cautious by proponents of the patch, was a setback not just to P&G but to several other companies whose products contain testosterone.

But safety isn't the only issue. Some researchers also worry that the new pills, patches, gels, and nasal sprays will lead women to take drugs for what are really social or psychological problems that can be treated more effectively with education or psychological intervention. "Most of women's sexual complaints have to do with self-respect, self-image, and the quality of the relationship," says clinical psychologist Leonore Tiefer of the New York University (NYU) School of Medicine, a leading critic of what she calls the "medicalization" of female sexuality. "They're things a pill can't treat."

"Our turn"

It's no surprise that the drug industry has turned its attention to women. Sildenafil, or Viagra—discovered when a candidate angina drug had surprising side effects—has grossed billions of dollars for Pfizer since its launch in 1998; copycats tadalafil (Cialis) and vardenafil (Levitra)—which also block the PD5 receptor, resulting in increased blood flow to the penis—have become successes in their own right. Meanwhile, interest in women's sexuality is growing; to wit, the adventures of four Manhattan women in the HBO smash hit series *Sex and the City*. "Women started asking: What's there for us?" says Harvard reproductive endocrinologist Jan Shifren, who directs the Vincent Menopause Program at Massachusetts General Hospital in Boston.

But exactly what sex drugs should do in women is much less obvious than in men. When the FDA put together "draft guidelines" in 2000 for companies interested in producing drugs to treat female sexual dysfunction (FSD), it followed the 4th edition of the *Diagnostic and Statistical Manual of Mental Disorders*. *DSM-4* says that FSD has four major components: decreased desire to have sex; decreased arousal (such as blood flow to the genitals and lubrication); pain during intercourse; and difficulty or failure to have orgasms. Companies must choose which component their drug affects and show efficacy in women with that problem.

Not everyone subscribes to that delineation, based on the classic model of the "human sexual response cycle" proposed by William Masters and Virginia Johnson in the 1960s. The distinction between desire and arousal, for instance, doesn't make much sense, says Ellen Laan, a sex researcher at the University of Amsterdam in the Netherlands. How to measure a drug's effect on people's sex lives is controversial, too. Because of those debates, the final version of FDA's guidelines has yet to appear.

In the meantime, about two dozen companies now have products in development for FSD (see table). Viagra, once considered a candidate to treat arousal problems, is no longer among them; Pfizer gave up last year after disappointing trials. But other companies have products that would do the same: Vivus in Mountain View, California, for instance, is in phase III trials with alprostadil, a vasodilating agent that women apply directly to their genitalia.

Most candidate drugs, however, focus on what clinicians say is by far the most common disorder: decreased interest in sex, also known as hypoactive sexual desire disorder (HSDD). Some of these compounds act on the central nervous system. One is flibanserin, a pill previously studied and rejected as an antidepressant, from German pharma giant Boehringer Ingelheim. Another, PT 141 from Palatin Technologies in Cranbury, New Jersey, is a nasal spray that stimulates melanocortin receptors in the brain.

Plain old testosterone is the basis for most of the desire-enhancing products. It usually comes in the form of skin gels, sprays, or patches, because the hormone is broken down quickly by the liver when taken orally. Women naturally produce testosterone, although at

CREDIT: GETTY IMAGES

Power patch. Both testosterone and a placebo improved sexual desire in the Intrinsa trials.

much lower levels than men, and production declines after menopause. Levels also drop on average by 50% after a woman's ovaries are removed, a condition called "surgical menopause." Several small trials suggested that testosterone enhances sexual desire in women, and U.S. doctors widely prescribe the hormone "off-label"—without being specifically approved—to women with HSDD. (European women are generally more reluctant to take hormones, Laan says.)

Because no specific product has been approved for women, doctors prescribe male testosterone drugs at about one-tenth of the dose or order pharmacists to produce special formulations that contain smaller amounts. A product aimed at and approved for women would be more convenient and safer, says Shifren, as well as opening a huge new market.

Those hopes were dealt a blow by the FDA panel in December. P&G had asked for approval of Intrinsa in surgically menopausal women first. The panel concluded that the results of two trials in this group were "clinically significant" if not exactly mind-blowing. (Roughly, patients who took placebo went from 3 to 4 "satisfying sexual episodes" per month, whereas those who got testosterone went from 3 to 5–5.5.) Side effects, such as increased facial hair growth and acne, were limited.

But the panel balked at the long-term safety data. The two trials together had enrolled 1095 women for 24 weeks—not nearly enough time to detect subtle risks resulting from long-term use, says panel member Steven Nissen, a cardiologist at the Cleveland Clinic in Ohio. Fresh on the panel's mind, he says, were the disturbing results of the Women's Health Initiative (WHI), a huge study funded by the U.S. government, which discovered in 2002 that long-term use of estrogen, alone or in combination with progestin, can increase women's risk of cardiovascular disease (see p. 1570). "We have a bad history with manipulating hormones in women," says Nissen, and the decision "wasn't even close."

Some critics say the vote smacks of a double standard, because drugs like Viagra, or even testosterone treatments in men, were never subjected to the long-term safety trials that the panel wished to see. "Be as conservative for men as you are for women," says Shifren. Clinical psychologist Sheryl Kingsberg of Case Western Reserve University in Cleveland, Ohio, calls the panel "very, very overconservative" and says it's "paternalistic" to deny women the choice to use testosterone.

The fate of Intrinsa and similar drugs is unclear. P&G withdrew its application after the panel meeting; a spokesperson says the company "is working with the FDA" to design new trials. "The key questions are going to be: How long does a trial have to be, and how many patients?" says Stephen Simes, president of BioSante Pharmaceuticals in Lincolnshire, Illinois, a company developing a testosterone gel. Companies will abandon their efforts if the agency requires studies like the WHI, which enrolled more than 16,000 women for 5 years in its main trial, Simes predicts. But Nissen says a trial of a few thousand women for 2 years, plus thorough postmarketing surveillance, might allay the worries.

Defining what's normal

But proving the safety of Intrinsa and its slew of competitors won't solve women's sexual problems, says NYU's Tiefer, who also gave a 3-minute presentation at the December meeting. Tiefer worries that women will feel compelled to start taking drugs, even if they're comfortable with their decreasing sex drives, once they become available. "I'm pro-sex," she says. "I'm pro-porn, I'm pro-vibrators. … But sex is a hobby. It's fine not to do it if you're not interested." (And certainly, an abusive husband like Laura's isn't a reason to put a woman on drugs, she adds.) Tiefer has founded a group, FSD Alert, that takes a feminist view of female sexual problems and puts more emphasis on sociocultural, political, and psychological factors.

There are other foes of FSD as a medical problem. In a series of articles over the past few years in the *British Medical Journal*, Ray Moynihan, a freelance journalist based in Sydney, Australia, called it the "corporate-sponsored creation of a new disease." He implicates the media for what he says are titillating but sloppy stories.

Those who favor the new drugs—even while admitting that they receive corporate support—dismiss this idea as absurd and slightly conspirational. Women had sexual problems long before drug companies started paying attention, says Shifren. And counseling or a getaway weekend with their partners, she notes, are some of many other options before medication. For some of her clients, lack of desire really is a source of misery, Shifren says.

Irwin Goldstein of Boston University adds that the critics are now telling women what men heard in the pre-Viagra era: that it's all in their heads. "They talk about medicalization. I call what they do psycholization," Goldstein says.

But even experts who believe that some women might benefit from medical treatment don't like the idea of large numbers of healthy women, nudged by wall-to-wall advertising on U.S. television, on FSD drugs. Already, the

Products for Female Sexual Dysfunction in Clinical Trials

DEVELOPING COMPANY	TREATMENT/MECHANISM	PROPOSED TRADEMARK	PHASE OF DEVELOPMENT
Acrus and Vivus	Testosterone transdermal spray	Testosterone MDTS	Phase II
Antares Pharma	Estradiol and testosterone patch		Phase I
BioSante	Testosterone gel	LibiGel	Phase III
Boehringer Ingelheim	Flibanserin. 5-HT_{1A} agonist and 5-HT_{2A} antagonist		Phase II
Cellegy	Testosterone gel	Tostrelle	Phase III
Columbia Laboratories	Intravaginal testosterone gel		Phase II
Eli Lilly	VML-670. 5-HT_{1A} agonist		Phase II
Galen Holdings	Intravaginal testosterone		Phase II
Nastech Pharmaceutical	Intranasal apomorphine		Phase II
NexMed	Alprostadil cream	Femprox	Phase II
NitroMed	NMI-870. Nitric oxide–enhanced α_2-agonist		Phase II
Novavax	Testosterone cream	Androsorb	Phase II
Palatin	PT-141. Melanocortin receptor agonist		Phase II
Procter & Gamble and Watson Laboratories	Testosterone patch	Intrinsa	Phase III
Retroactive Bioscience	Topical nitric oxide induction/lubricant	Sensua!	Phase II/III
Sepracor	Didesmethylsibutramine		Phase I
Solvay	Estrogens and methyltestosterone	Estratest	Phase III
Vivus	Topical alprostadil	Alista	Phase III

CREDITS (TOP TO BOTTOM): PHOTO RESEARCHERS INC.; SOURCE: M. BRUNO, M. FELLER, AND W. SIETSEMA/*GOOD CLINICAL PRACTICE JOURNAL* 12, PJB PUBLICATIONS (2005)

extent of the problem is being blown far out of proportion, says John Bancroft, a former director of the Kinsey Institute at Indiana University, who is now retired in England.

For instance, a 1999 study by Edward Laumann and his colleagues at the University of Chicago found that a staggering 43% of women between 18 and 55 suffer from sexual dysfunction—a number often repeated in scientific literature and the press that Bancroft calls "extreme." A recent British study suggests that many problems are transient, he adds: Although 40% of women reported having a problem with sexual function that lasted at least 1 month, the study found, only 10% had complaints that lasted longer than 6 months.

Laan, at the University of Amsterdam, also believes that there's nothing medically wrong with most of the women who have arousal or desire problems. Instead, she says they just need more sexual stimulation. A recent German study among college students, for instance, showed that a woman's desire dropped with the duration of the relationship. "It's a huge taboo to say so, but many women who have lost interest in their partner still feel like having sex with the guy next door," Laan says. But desire can be stimulated, she adds, by anything from romantic dinners to fantasizing: "It's just something that takes some work."

Ironically, the drug trials themselves suggest that some women may not need desire-boosting drugs. Most show a considerable placebo effect; in the Intrinsa studies, for instance, some 36% of patients on placebo wanted to continue after the study closed. Maybe P&G should just market the placebo, Nissen quipped during the panel meeting. Talking about a sexual problem and deciding to tackle it might have a therapeutic effect by itself, say researchers.

Even with all the questions about FSD drugs, Bancroft believes that the increased attention will benefit the field. "We're having a very healthy debate," he says. "The good thing is that we'll come out of this with a much better understanding of women's sexuality."

–MARTIN ENSERINK

NEWS

Bone Quality Fills Holes in Fracture Risk

Osteoporosis isn't the only factor behind broken bones. A better understanding of bone quality, coming from biochemical markers and refined imaging techniques, will help predict who is most at risk of debilitating fractures

When a woman is tested for osteoporosis, technicians shoot low-dose x-rays through her hip to get a picture of the bone and a measure of its density. The less bone, the higher the overall risk of breaks, including debilitating hip fractures. But over the last decade, researchers have come to a greater awareness that it's not just quantity that matters: Bone quality counts for a lot.

The importance of bone quality—a term covering aspects such as the organization of the tiny struts that make up the inner tissue—became obvious during clinical trials of drugs for osteoporosis. These drugs prevent the loss of bone, but it turned out that, statistically, bone mineral density (BMD) couldn't explain all of the reduction in fracture risk. That fit with observations by clinicians: Some women with osteoporotic bones don't suffer breaks, whereas many women with apparently healthy bones still end up with fractures.

Identifying women at risk before they fracture is "the most challenging public health question" facing osteoporosis researchers, says Ego Seeman of Austin Hospital in Melbourne, Australia. And it's not just an issue for women. Osteoporosis is becoming more common in men, and more commonly diagnosed, especially as they live longer.

Researchers are trying to get a better handle on bone quality in several ways. They're searching for new and better biochemical markers of bone change, to add to the handful already used in the clinic to assess the effects of drugs. Higher resolution imaging with computed tomography (CT) and magnetic resonance imaging (MRI) is beginning to probe the inner architecture of bones without the need for direct sampling.

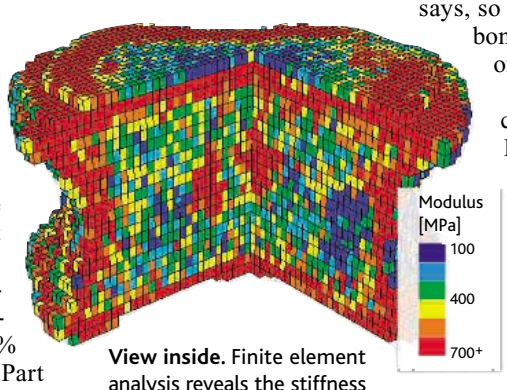
The hope is that these advances may one day better identify patients in need of treatment, as well as provide a way to chart their progress on drugs, but the newer imaging techniques are still being developed and won't be widely available for several years. In the meantime, some researchers are trying to integrate proven risk factors to predict a woman's chance of fracture.

Strong bones

Osteoporosis is a factor in more than 1.5 million fractures each year in the United States alone. Costs have been estimated at more than $17 billion a year, particularly from hip fractures, more than 75% of them in women. Part of the reason is that women who are not in nursing homes are twice as likely as men to fall, perhaps because they lose muscle strength faster with age. But another major factor is that their bones tend to become much weaker with age than men's do.

Strength comes from two features of bones. The outer shell of dense material, called cortical bone, is like the metal tubing of a bicycle that makes a strong, light frame. Inside this cortex is a porous network of tiny support struts and rods, called trabeculae. Trabecular bone makes up just 20% of bone mass but most of its surface area.

Sex differences appear relatively early in life. Growing girls tend to add more mass to the inner side of the bone cortex, beefing up the trabeculae to create a storehouse of calcium for pregnancy and lactation. Boys, in contrast, tend to add more material to the outside of the cortex. The greater the diameter, the stronger the bone. The effect, as seen in cross-sectional studies, is "absolutely huge," says Heather McKay of the University of British Columbia in Vancouver. In addition, girls tend to be less active than boys, she says, so many don't get the bone-building benefits of exercise.

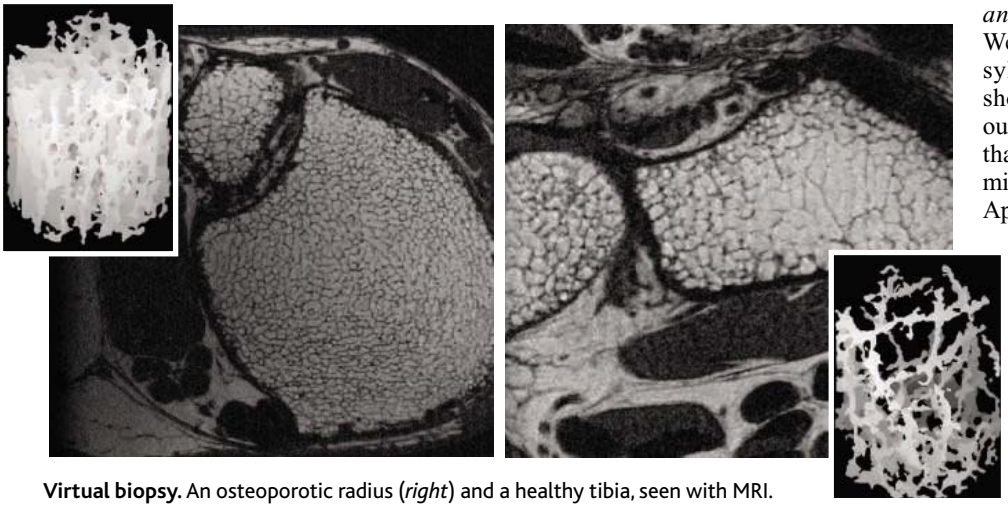


View inside. Finite element analysis reveals the stiffness of bone, such as this vertebra with low mass.

The big kicker comes at menopause. Estrogen is a key regulating signal for the cells that are constantly remodeling bone, thus repairing damage and allowing bones to bulk up to the loads placed on them. When estrogen levels decline during menopause, the bone-building cells known as osteoblasts slacken their activity. But the bone-resorbing osteoclasts continue to remove bone mineral and break down collagen. That means women typically lose 1% to 2% of their bone per year around menopause, more of it from trabecular bone.

CREDIT: IMAGE COURTESY OF DANNY LEUNG AND JENNI BUCKLEY/UC BERKELEY

Several risk factors influence the likelihood that a woman will lose more bone than normal and eventually suffer a fracture. A previous fracture ups the odds substantially, as does a family history of fracture, although genetic factors remain fairly murky. Race matters, too. The incidence of hip fractures is 25% lower in Asian than in white women, for example, even for women with similar bone densities. Behaviors—poor diet and lack of exercise, especially in youth—are also negative influences on bone health, as discussed in a massive report from the Surgeon General last year.*



Virtual biopsy. An osteoporotic radius (*right*) and a healthy tibia, seen with MRI.

CREDITS: FELIX W. WEHRLI/UNIVERSITY OF PENNSYLVANIA

These risk factors are fairly weak predictors of an individual's absolute risk, however. Up until the 1980s, clinicians basically waited until a fracture occurred before treating patients for osteoporosis. Diagnosis—and research—got a considerable boost in the 1990s with the advent of dual x-ray absorptiometry (DXA). "It just revolutionized the field," says B. Lawrence Riggs of the Mayo Clinic in Rochester, Minnesota. DXA enabled clinicians and researchers to follow patients over a long time and assess their responses to medications, helping bring the current crop of drugs to market, Riggs says (*Science*, 3 September 2004, p. 1420). In the United States, the National Osteoporosis Foundation recommends that women over the age of 65, or younger women who have one or more risk factors, be tested with DXA for osteoporosis.

But DXA's usefulness for making predictions is limited. "The number one clinical goal is to be able to sit down with a patient and give a numerical indicator of fracture risk," says Lawrence Raisz of the University of Connecticut Health Center in Farmington. DXA doesn't do that well, although many researchers point out that it's a better predictor than is cholesterol level for heart disease. By factoring in bone quality as well, researchers and doctors eventually hope to do better.

Sharpening the picture

One of the main approaches to gleaning details about the quality of bones is to measure the activity of osteoclasts and osteoblasts, the cells that remodel bone and thus influence its structural properties. The first cell activity marker approved by the U.S. Food and Drug Administration, in 1995, measures the products of bone breakdown and can pick out women with extremely high rates of bone loss. In general, however, markers are not currently useful for diagnosis of osteoporosis, because levels overlap between those who have and don't have the disorder. Researchers are trying to explain the variability and investigating new markers that might be more specific.

The main clinical use of markers at the moment is to help chart how patients respond to drugs. That kind of information may also encourage patients to keep taking their medicine, as Pierre Delmas of Claude Bernard University in Lyon, France, explained last month at a meeting on bone quality run by the National Institutes of Health and the American Society of Bone and Mineral Research in Bethesda, Maryland. His unpublished data showed that providing patients with progress reports from biomarkers could increase the numbers who stay on their medications by 20%. Biochemical markers may also help refine the assessment of fracture risk, but the results of large studies so far have been inconsistent.

Another way of getting new information about bone quality is by looking at bone architecture directly. A time-tested research method is to study actual bone from biopsies, cadavers, or hip replacement operations. CT and electron microscopy can resolve individual rods and struts, the crucial support elements inside trabecular bone. But direct sampling is too invasive and expensive to be used to track individual patients' health.

Researchers have been trying to get similar and more clinically useful information using imaging tools. One benchmark in the field is a 2001 paper in the *Journal of Bone and Mineral Research* (*JBMR*) by Felix Wehrli's group at the University of Pennsylvania, Philadelphia. The researchers showed in a study of 79 women with various bone densities and vertebral deformities that a souped-up MRI machine can reveal microscopic bone structure noninvasively. In April, a group led by Charles Chesnut of the University of Washington, Seattle, published online in *JBMR* the first such longitudinal study of bone microarchitecture with MRI.

The other main imaging techniques use quantitative CT, mainly to study peripheral bones, such as the forearm. Aspects of bone quality are then extrapolated to hip and spine. Given the small size of studies so far, CT and MRI haven't been used to assess fracture risk. Researchers say those results should come in the next few years: Larger trials are incorporating CT and MRI in subsets of patients.

One attempt to get at fracture risk is already under way. Tony Keaveny, a biomechanical engineer at the University of California, Berkeley, is using a technique called finite element analysis. Keaveny and colleagues take CT images of human vertebrae, including information about the trabecular architecture, and model how they respond to stress. In a paper published in *Bone* in 2003, he and his former student R. Paul Crawford showed that their analysis of CT images of cadaver bones predicted 85% of the variation in bone strength in experiments with actual loadings of the bones—"better than BMD did," he says. Ultimately, Keaveny says, the method should be able to provide a personalized fracture risk assessment for patients, adjusted for their height and weight and other factors. Clinicians say the approach is exciting but might be prohibitively expensive for screening patients.

In the meantime, clinicians and researchers say much can be done to get more women checked for osteoporosis and give patients a better idea of their fracture risk. In one high-profile effort, a center at the University of Sheffield, U.K., sponsored by the World Health Organization has been designing a method to express a person's absolute risk of fracture during the next 10 years. "This will allow us to have a standard of care," comments Ethel Siris of Columbia University College of Physicians and Surgeons. "It will give us a better threshold for determining treatment."

—ERIK STOKSTAD

* *Bone Health and Osteoporosis: A Report of the Surgeon General* (2004), HHS. www.surgeongeneral.gov/library/bonehealth/content.html

REVIEW

HIV/AIDS in Women: An Expanding Epidemic

Thomas C. Quinn[1,2*] and Julie Overbaugh[3]

More than 20 years into the human immunodeficiency virus–type 1 (HIV-1) epidemic, women account for nearly half of the 40 million people living with HIV-1 worldwide, with an even higher proportion existing in developing countries. Social determinants of female vulnerability to HIV-1 include gender disparities, poverty, cultural and sexual norms, lack of education, and violence. Women are also more susceptible to HIV-1 because of hormonal changes, vaginal microbial ecology and physiology, and a higher prevalence of sexually transmitted diseases. Prevention strategies must address the wide range of gender inequalities that promote the dissemination of HIV-1.

For the past two decades, HIV-1 infection and its consequent disease, acquired immunodeficiency syndrome (AIDS), have affected more women worldwide than any other life-threatening infectious disease. Women account for nearly half of the 40 million people living with HIV-1 (Fig. 1A) (*1*). In sub-Saharan Africa, females constitute 60% of those infected with HIV-1 (Fig. 1B) and 75% of infected individuals between the ages of 15 and 24 (*2*). In South Africa, Zambia, and Zimbabwe, young women aged 15 to 24 are three to six times more likely to be infected than young men. In South Africa, one in four women is HIV-1–infected by the age of 22. Women make up half of the adults living with HIV-1 in the Caribbean, and one-third in Latin America, with a higher burden in young women (*1*). In addition to the direct impact that HIV-1 infection has on these women, there is also the known high risk of HIV-1 transmission to their infants and a resulting plethora of consequences for the family.

In the United States, the annual number of estimated AIDS cases increased 15% among women and only 1% among men from 1999 to 2003. The major burden of disease was in young women and women of color, particularly African-American and Hispanic women, who often have reduced access to health care (*3*). The rate of AIDS diagnoses for African-American women was approximately 25 times the rate for white women and four times the rate for Hispanic women. The majority of infections were due to heterosexual transmission (80%) or to injecting drug use (19%). These same risk factors, especially injecting drug use, have led to a 50% increase in infections in women in Asia and eastern Europe over the past 2 years (*1*, *2*).

Physiologically, women are more susceptible to HIV infection than are men. Increased susceptibility among women has been linked to specific cofactors, including the use of hormonal contraceptives and the increased presence of sexually trasmitted diseases (STDs) (*4*, *5*). The role of hormonal contraceptive use in increasing HIV-1 infection in women has been controversial, with one longitudinal study showing an association (*5*) but another not showing an effect (*6*). Hormonal contraceptive use has been linked to the infection of women with several viral strains from their partners, which in turn may accelerate disease progression (*4*). The role of hormonal contraceptives remains a key issue, given the common use of hormonal methods

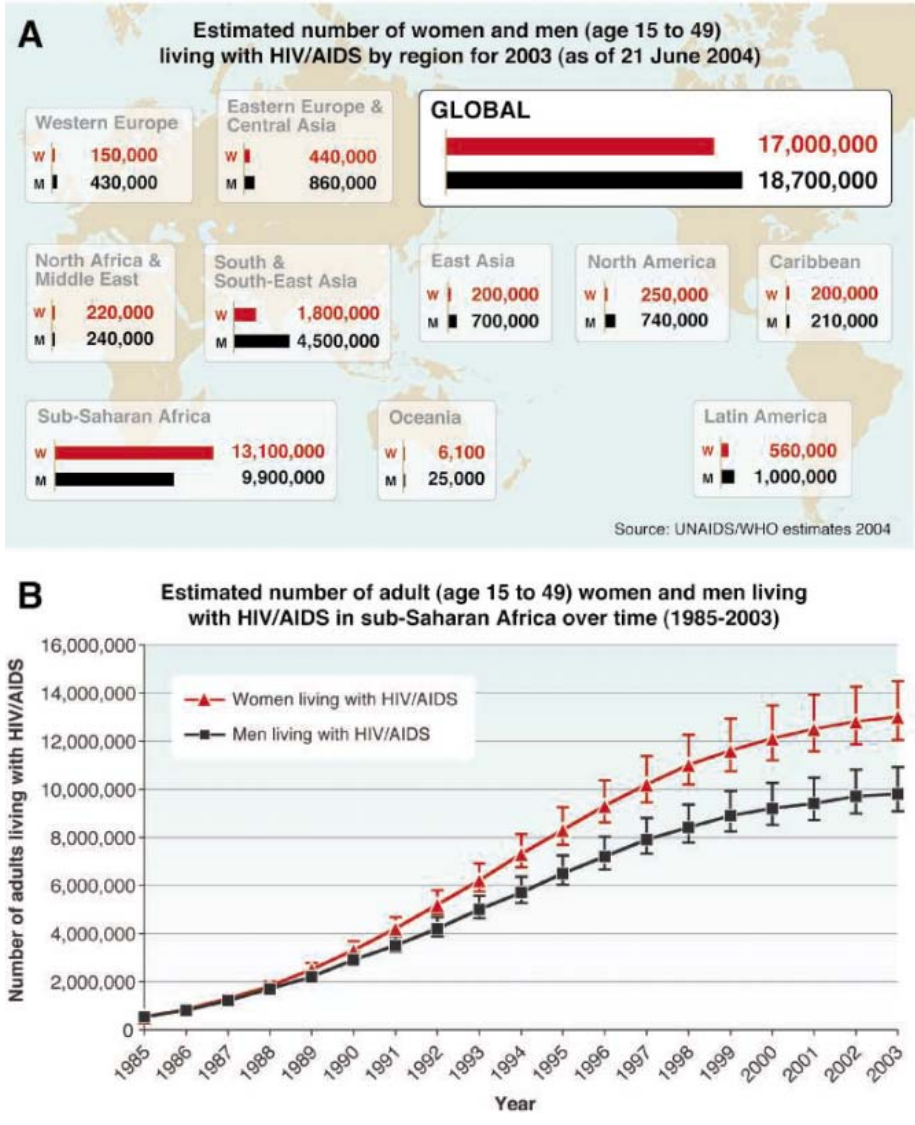


Fig. 1. (**A**) Estimated number of adult (age 15 to 49) women (red) and men (black) living with HIV/AIDS by region from 1985 to 2003 (*1*). (**B**) Estimated number of adult (age 15 to 49) women (red) and men (black) living with HIV-1 in sub-Saharan Africa from 1985 to 2003 (*2*). [Reproduced by permission of UNAIDS, the Joint United Nations Programme on HIV/AIDS.]

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of contraception in at-risk populations, such as young females.

Much of our inability to define the biological role of these cofactors in HIV-1 susceptibility comes from a lack of understanding of the initial events in HIV-1 infection (*7*). HIV-1 is transmitted to women primarily via heterosexual contact, and the virus must therefore penetrate the mucosal barrier to establish a systemic infection. Ulcerative STDs increase the risk of HIV-1 infection, which implies that breaches in mucosa enhance HIV-1 transmission. Additionally, STDs increase the presence of inflammatory cells, a result that provides more potential targets for the virus. Fundamental questions regarding the biology of HIV-1 transmission have been difficult to answer, because it is hard to examine relevant cells and tissue at the time of HIV-1 acquisition, when the initial pivotal events are occurring.

Susceptibility to HIV-1 also varies throughout a woman's reproductive life. Adolescent girls appear to be the population most vulnerable to HIV-1, either because of behavioral high-risk activities or because of the physiological properties of an immature genital tract with increased cervical ectopy or exposed columnar epithelium. In addition, recent studies have shown a twofold increase in the risk of HIV-1 acquisition during pregnancy and the early postpartum period, even after adjustments have been made for changes in sexual behavior and social and demographic factors (*8*). Factors that could increase susceptibility during pregnancy include high levels of progesterone, which has been shown to enhance susceptibility in nonhuman primate models of HIV-1 (*9*), and increased ectopy. The mechanisms by which female hormones may affect HIV-1 susceptibility include increases in the number of target cells and the suppression of immune responses, but these mechanisms remain poorly defined.

If hormonal changes play a key role in HIV-1 susceptibility and the magnitude of the immune response to infection, then it is critical that vaccine trial design consider possible gender differences in outcome. Indeed, some preliminary findings from the only phase-III HIV-1 vaccine trial conducted to date suggested that there may be differences in the humoral immune responses to the vaccine generated in women and men (*10*). However, this difference was not detected in an analysis of smaller phase-I/II vaccine trials (*11*).

This growing "feminization" of the HIV-1 pandemic reflects women's greater social and biological vulnerability (*12*). Because gender norms shape attitudes toward information on sex, sexuality, sexual risk-taking, and fidelity, they play a critical role in determining the course of the epidemic. Because the risk of HIV-1 infection in women has been linked to the regional norms that affect power in interpersonal relationships (*12*), controlling the HIV-1 pandemic requires intensive attention to gender-related issues driving the epidemic. Interventions must be multifaceted and should include making both female and male condoms accessible to all in ways that do not stigmatize; prioritizing the development of female-initiated methods of protection such as microbicides; defining the influence of hormones on disease progression and response to treatment; and educating women and men about HIV-1 and other STDs, including how to negotiate safe sex, and encouraging them to seek testing and treatment.

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REVIEW

Molecular and Cellular Basis of Cardiovascular Gender Differences

Michael E. Mendelsohn* and Richard H. Karas

Cardiovascular diseases (CVDs), the major cause of morbidity and mortality for both men and women, occur uncommonly in premenopausal women, but their incidence rises sharply after the menopausal transition. Cardiovascular gender differences are apparent long before CVDs appear in men and women, and improved understanding of the biology underlying these differences has the potential to advance the diagnosis and treatment of CVDs in both sexes. This review considers gender differences in the molecular and cellular physiology of the heart and blood vessels in health and disease, highlighting understudied areas that can help resolve the current controversy regarding hormone replacement therapy and improve cardiovascular health in women.

Sex Steroid Hormones, Receptors, and Gender Differences

Women develop heart disease later in life than men. This difference has been attributed to the loss of female sex steroid hormones at the time of menopause, but the biological explanations for gender differences in cardiovascular diseases (CVDs) are more complex. Recent advances in research on cardiovascular gender differences have increased our understanding of the biology responsible; however, a synthesis of the underlying mechanisms that explain these differences has not yet been possible. The current controversy that has arisen from the Women's Health Initiative (WHI) trials of the cardiovascular effects of hormone replacement therapy (HRT) on CVD (*1*) is a case in point. This controversy is in part due to an underappreciation of the relationship between the timing of HRT initiation and differences in the underlying vascular biology that exist between perimenopausal and older women. Resolving this controversy will require a more complete understanding of the molecular and cellular physiology of each of the sex steroid hormones and their receptors in the cardiovascular system and a greater focus on how the extent of underlying atherosclerosis affects the response to HRT.

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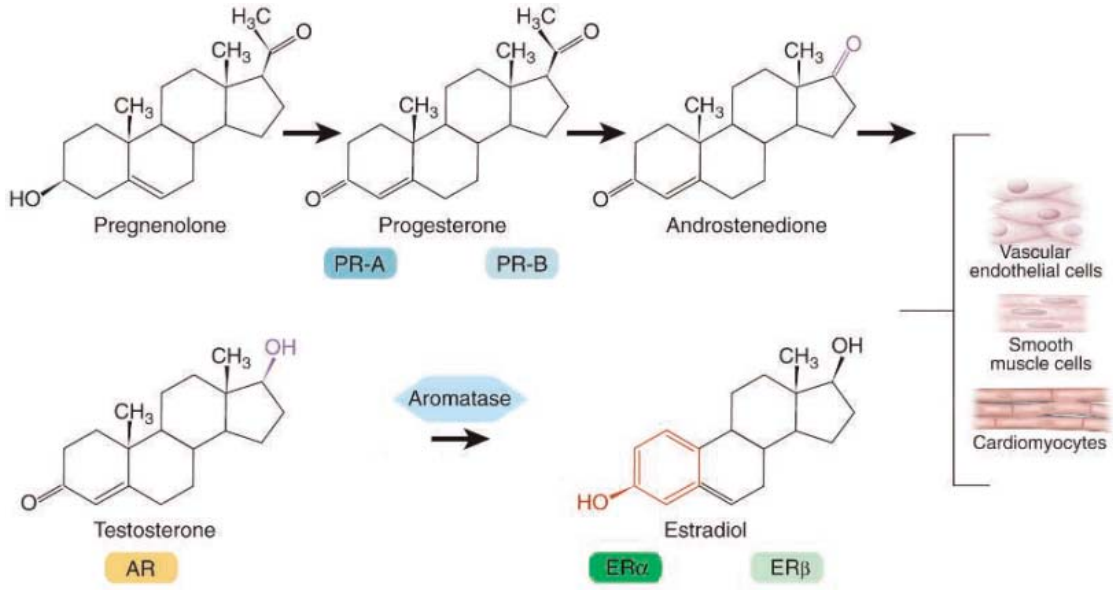

Fig. 1. Sex steroid hormones and sex steroid hormone receptors. Synthesis of the three gonadal sex steroid hormones, estrogen, progesterone, and testosterone, from pregnenolone. Pregnenolone gives rise first to progesterone, which serves as the intermediate for the synthesis of androgens and estrogens. Estrogens are synthesized from androgens by the formation of an aromatic A ring, which is catalyzed by the enzyme aromatase (Cyp19). Steroid hormones bind to and activate specific members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. All of the sex steroid hormone receptors, and the enzyme aromatase, are expressed in vascular endothelial cells, vascular smooth muscle cells, and cardiomyocytes (right).

Sex steroid hormones (SSHs) and their receptors are critical determinants of cardiovascular gender differences. Most research has focused on the effects of estrogen and estrogen receptors (ERs) on cardiovascular physiology and disease, whereas progesterone and testosterone and their receptors (PR and AR), and the enzyme aromatase, which converts testosterone to estrogen in specific tissues (Fig. 1), have received far less attention. Steroid hormones activate their cognate receptors, members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. Ligand-bound sex steroid hormone receptors (SSHRs) dimerize and bind to specific DNA response elements, and engage the general transcriptional apparatus, as reviewed previously (*2*, *3*). Several newer SSHR signaling concepts with implications for cardiovascular physiology have emerged recently (Fig. 2, points 1 to 6).

First, steroid hormone receptors do not act alone, but interact with a broad array of coregulatory proteins to alter transcription (Fig. 2, point 1). Cell-specific expression of coactivator and corepressor proteins and their regulation by post-translational modifications allow for exquisite tissue-specific and temporal regulation of SSHR-mediated transcription (*2*, *4*). Understanding coregulator biology is important to the development of cardiovascular-selective estrogen receptor modulators (SERMs) and modulators for other SSHRs. Examples of cardiovascular coregulator specificity include the role of the ER coactivator protein steroid receptor coactivator 3 (SRC3) in mediating estrogen inhibition of vascular injury (*5*) and the relatively specific myocardial AR coactivator, FHL2 (*6*). However, little is known about the differential expression and function of coregulatory molecules in myocardial and vascular cells. SSHRs also participate in rapid cellular activation pathways that at least initially do not alter gene expression (Fig. 2, point 2), such as rapid stimulation of vascular endothelial nitric oxide synthase (NOS) and vascular dilatation mediated by activated ER (*3*, *7*, *8*). Rapid signaling pathways can also converge on genomic pathways, activating transcription of genes lacking SSH response elements (*8*, *9*). ER and PR in vascular cells also can be activated in the absence of ligand by growth factor pathways (*3*, *8*) (Fig. 2, point 3). In addition, SSHRs cross-regulate expression of one another in vascular cells (*8*, *10*) (Fig. 2, point 4). These pathways all add substantial combinatorial complexity to the physiological effects of SSHs in target tissues. Genetic SSHR variants influence individual responses to SSHs (Fig. 2, point 5) and are associated with altered cardiovascular risk in both sexes (*11*, *12*), but the physiological consequences of such variants are unexplored. Finally, SSHRs can regulate non-SSH nuclear receptors such as the peroxisome proliferator-activated receptor α (PPARα), and the liver X receptors (LXRs), which govern metabolic pathways directly relevant to CVD (*13*, *14*) (Fig. 2, point 6). These newer concepts of SSHR action (Fig. 2) require greater attention in all target tissues, including the cardiovascular system.

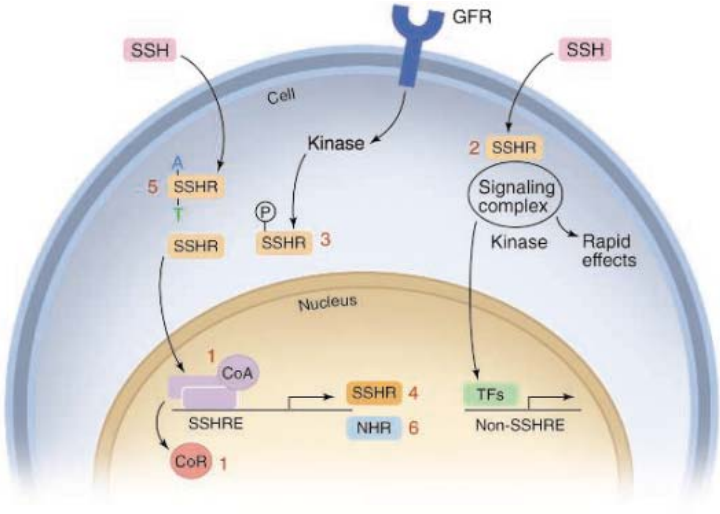

Fig. 2. Emerging concepts in sex steroid hormone receptor signaling of potential importance in cardiovascular physiology. Newer concepts in SSHR action that are relatively unexplored in cardiovascular cells and tissues are depicted (see text). Abbreviations: GFR, growth factor receptor; SSHR, sex steroid hormone receptor; SSHRE, SSH response element; CoA, coactivator; CoR, corepressor; NHR, non-SSHR nuclear receptors; TFs, transcription factors.

Gender Differences in Blood Vessels

Vascular SSHR and aromatase. The expression of SSHRs and aromatase in the vasculature is well recognized (*3*, *15*), but how the expression of these proteins in cardiovascular cells varies with gender, vascular bed, and the presence of car-

diovascular risk factors or CVD is unclear. AR and the two PR isoforms (PR-A and PR-B) are expressed in the vasculature, but little is known about their functions in cardiovascular physiology. In mouse models, ERα mediates most of the protective effects of estrogen on injured blood vessels, including promoting reendothelialization (*16*) and inhibiting smooth muscle cell proliferation and matrix deposition following vascular injury (*17*), and attenuating atherosclerotic plaque progression (*18*, *19*). ERα-mediated protection in low density lipoprotein (LDL) receptor–deficient female mice is in part due to E2-ERα–dependent production of the atheroprotective molecule prostacyclin (*19*).

Studies of cardiovascular gender differences have focused mainly on later life stages, but mammalian hypothalamic-pituitary-gonadal axis function begins in utero, when testosterone is first produced (*20*). Ovarian endocrine activity begins shortly after birth, and estradiol (E2) levels are significantly higher in prepubertal girls than in boys (*20*, *21*). At puberty, gonadotropin secretion rises, stimulating gonadal SSH production. The cyclic variation in estrogen and progesterone then continues for nearly four decades in women, when the perimenopausal transition begins. The need to study cardiovascular gender differences during premenopausal years and the perimenopausal transition is now gaining attention (*22*).

Vascular tone and blood pressure. Hormone-dependent gender differences exist in vascular function. Estrogens cause vasodilatation through both rapid increases in NO production and induction of NOS genes (*3*, *8*, *23*). Blood pressure is lower in adolescent and premenopausal women than in age-matched men, and rises following menopause (*24*, *25*). Vasodilatation and blood pressure are both affected by fluctuations in circulating estrogen levels during the menstrual cycle, pregnancy, or E2 supplementation (*24*–*26*). In men, short-term estrogen administration has little effect on vascular relaxation, whereas longer-term administration improves vasodilatation (*25*). HRT does not lower blood pressure to premenopausal levels, suggesting a role for unopposed androgens in blood pressure regulation following menopause (*26*). Progesterone lowers blood pressure, whereas synthetic progestins can raise blood pressure (*24*). ERβ is required for normal vasodilatation and blood pressure in both males and females, with loss of ERβ causing more substantial hypertension in males (*27*).

Lipids. Lipid abnormalities contribute substantially to atherosclerosis and are regulated both by SSH and HRT, principally by way of hepatic effects on lipoprotein metabolism [reviewed in (*3*, *28*, *29*)]. The liver expresses ERα, PR, and AR, but not ERβ, which influences cardiovascular effects of HRT formulations and SSHR modulators. In clinical and animal studies, E2 inhibition of atherosclerosis is only partly explained by lipid changes (*3*, *30*). After menopause, LDL and triglyceride levels rise, and high density lipoprotein (HDL) levels fall. HRT has antiatherogenic effects on lipids, lowering LDL and raising HDL (*3*, *28*, *29*), but paradoxically also elevates triglycerides. HRT alters hepatic synthesis and/or clearance of many lipoproteins (*3*, *29*). In both male and female apolipoprotein E–deficient mice, E2 inhibits atherosclerotic lesion formation in a manner not fully explained by changes in lipids (*30*). Testosterone's effects on lipids are discussed below.

Hemostasis and thrombosis. HRT causes an increase in venous thromboembolic events, but the effects of SSH on coagulation, fibrinolysis, and arterial thrombosis are understudied (*28*, *31*). Oral HRT and contraceptives increase levels of Factor VII, but decrease circulating fibrinogen and plasminogen activator inhibitor–1 (*31*). Genetic variants like the common variant Factor V Leiden may predispose to thrombosis in the setting of HRT (*12*, *31*). Megakaryocytes express ERβ and AR, but not ERα or PR (*32*). Platelet aggregation and secretion change with sexual maturity differently in females and males (*33*). Further studies are needed of coagulation/fibrinolysis and platelet function in gonadectomized animals, mice

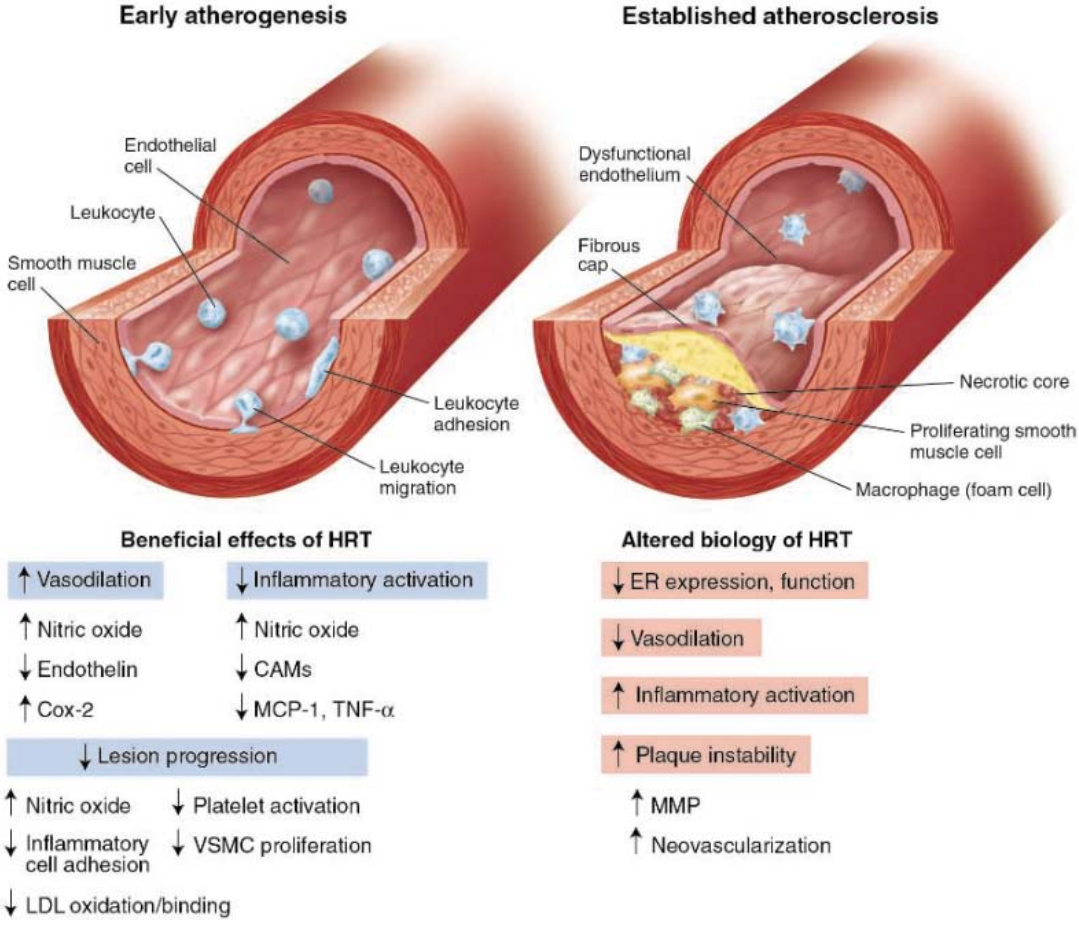

Fig. 3. The timing hypothesis: differential effects of HRT on early and later stages of atherosclerotic disease. Atherosclerosis is characterized by the gradual loss of vascular protective mechanisms and the emergence of advanced, unstable lesions (*38*). SSH effects on the endothelium and its protective functions, vascular smooth muscle cells, and inflammatory cells differ, depending on the stage of atherosclerosis in the underlying blood vessel (*3*, *8*, *24*, *35*, *39*, *40*). LDL, low density lipoprotein; CAMs, cell adhesion molecules; MCP-1, monocyte chemoattractant protein 1; TNF-α, tumor necrosis factor–α; VSMC, vascular smooth muscle cell; MMP, matrix metalloproteinase; COX-2, cyclooxygenase 2.

harboring SSHR disruptions, and humans on HRT.

Evolution of atherosclerosis and the timing of HRT. Observational studies consistently show that CVD risk decreases with HRT use and increases with premature menopause (*1*, *3*, *29*), supporting evidence that estrogen/progesterone loss and/or unopposed androgen promotes postmenopausal CVD. In contrast, the WHI and other randomized trials of HRT fail to show an HRT effect in lowering cardiovascular events (*1*). This discordance remains widely misinterpreted as strong or definitive evidence that HRT does not afford cardioprotection. Both methodological and biological issues contribute to the differences between observational and randomized clinical trials (*34*), but the age at which women initiate HRT is likely critical. Studies in primates (*35*) and other animal models (*36–38*) support evidence that the beneficial effects of HRT in preventing atherosclerosis occur only if HRT is initiated before the development of advanced atherosclerosis (the timing hypothesis) (Fig. 3). Atherosclerosis is a complex, progressive inflammatory process characterized by the gradual loss of vascular atheroprotective mechanisms and the emergence of susceptibility to plaque instability and rupture (*39*, *40*). The timing hypothesis states that SSHs alter the biology of vessel wall cells and the inflammatory cells that accrue as atherosclerosis progresses differently in the early versus later stages of the disease (Fig. 3). Early, physiological levels of SSH replacement can improve or reverse the endothelial dysfunction that occurs before the development of more advanced atherosclerotic lesions [reviewed in (*3*, *8*, *24*, *35*, *40*)] (Fig. 3). In advanced atherosclerotic lesions, however, a different cellular biology exists that provides an altered substrate, which in response to the late initiation of HRT is more susceptible to inflammatory and hemostatic abnormalities (Fig. 3).

Failure to account for timing data contributes to the present confusion in interpreting trials of the effect of HRT on CVD. Most women in observational studies initiated HRT during the perimenopause (*41*), whereas the WHI trial included too few younger women to examine whether women starting HRT during the menopausal transition achieve cardioprotection (*42*). There are proven therapies for CVDs in women that continue to receive insufficient attention and use in the midst of this controversy, such as HMG CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors (statins), which prevent and reverse atherosclerosis and decrease cardiovascular events (*43*). Preclinical data supporting the timing hypothesis have not yet been adequately considered and incorporated into clinical trial study designs. Two prospective clinical studies to directly address the HRT timing hypothesis are currently enrolling patients: the Kronos Early Estrogen Prevention Study (KEEPS) and the Early versus Late Intervention Trial with Estradiol (ELITE).

Cardiovascular effects of testosterone. In both sexes, testosterone levels decline with age, but at a more gradual rate than estrogen and progesterone decreases in women following menopause. In men, circulating estrogen levels are quite low, but appear to be physiologically relevant, because estrogen deficiency in males increases testosterone levels (*44*). In male mice, testosterone inhibition of atherosclerosis is abrogated by aromatase inhibition (*45*). Aromatase inhibitors disrupt normal vascular relaxation in healthy human males, and aromatase knockout mice have abnormal vascular relaxation, supporting evidence that conversion of testosterone to estrogen in males by aromatase helps maintain normal vascular tone [reviewed in (*46*)]. However, effects of aromatase inhibitors on vascular function in females have not yet been examined, despite their widespread use in breast cancer therapy. Androgen replacement therapy (ART) is controversial in both sexes, but in general is not associated with increased cardiovascular risk (*47*, *48*). Several ART studies suggest a beneficial cardiovascular effect, especially on vasomotion (*47–50*). ART can improve cardiac ischemic indices in men, but not ischemia caused by peripheral arterial disease (*49*). Androgens have variable effects on lipoproteins and other risk factors, depending on the hormone formulation used and population studied. Exogenous androgens generally lower HDL-C and lipoprotein (a) [Lp(a)], with only modest effects on LDL-C (*50*), and facilitate both macrophage lipoprotein uptake and efflux of cellular cholesterol to HDL (*50*). ART in men with coronary artery disease enhances coronary blood flow and endothelial function (*51*). Testosterone activates both AR and ER (by aromatase conversion to E2) in cardiovascular tissues, but the relative importance of ER and AR for vascular androgen effects and the specific cardiovascular genes regulated by these receptors are not yet known. Normal ER function is required in both males and females for normal cardiovascular development and function (*27*, *44*, *46*, *52*). A man lacking functional ERα has impaired vascular function and early coronary arterial calcification (*52*). Although interest in the potential for ART in both sexes has increased in recent years (*47*, *48*), randomized trials of ART in either gender are lacking (*47–50*).

Gender Differences in the Heart

SSHR and aromatase all are expressed in the heart (Fig. 1) [reviewed in (*3*, *15*, *53*)]. Recent controversy has arisen as to whether murine hearts express ERβ (*54*), but functional ERα and ERβ have been detected in animal and human cardiomyocytes (*3*, *15*, *53*). Gender differences exist in normal heart function. Cardiac contractility is greater in healthy women than in age-matched men, and HRT withdrawal in women decreases contractility (*55*, *56*). As men and women age, myocardial mass is better preserved in women (*57*), which may be related to differences in cardiac expression of glycolytic and mitochondrial metabolic enzymes (*58*) and/or to prosurvival effects of E2-ER on cardiomyocytes mediated by ERα- and phosphatidylinositol 3-kinase–Akt–dependent pathways (*59*).

Gender differences also exist in cardiac electrophysiological function [reviewed in (*60*)] and in both inherited and acquired abnormalities of the heart muscle. Some familial hypertrophic cardiomyopathies are more severe in males than in females (*61–63*). Hearts of women with aortic stenosis are more hypertrophied and have better contractile function than those of men with this disorder (*64*). In heart failure studies, female gender is associated with improved cardiac function and survival (*65*, *66*). Animal studies of ischemia and reperfusion injury show that female gender confers protection, which requires ERβ (*67*). Estrogen benefits but testosterone worsens cardiac function in a mouse model of myocardial infarction in both males and females (*68*), suggesting that myocardial ER and AR may mediate opposing effects on the myocardial response to injury. Further study of these two SSHRs in normal and diseased myocardium of peri-and postmenopausal women is needed. SSHR-mediated changes in the levels and regulation of myocardial calcium-contractility coupling proteins in the heart are likely involved in the effects of SSH on myocardial hypertrophy and heart failure (*69*, *70*) and also need greater study.

Summary

New, better tailored hormone replacement therapies and selective SSHR modulators of use in preventing and treating CVD are needed. To improve diagnosis and treatment of CVD in women, it will be necessary to strengthen interactions between preclinical and clinical scientists, improve our understanding of the biology of gender differences and the perimenopause, and reconsider the paradigm of and singular focus on untailored postmenopausal HRT that has dominated the past several decades. To accomplish these goals, greater focus on understanding the molecular and cellular physiology of each of the SSHs and their receptors in the cardiovascular system will be required.

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71. We regret that strict space limitations necessitated frequent citation of reviews and prevented the referencing of many original studies and recent interesting work.

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SPECIAL SECTION

REVIEW

The Pains of Endometriosis

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Endometriosis is a disease defined by the presence of endometrial tissue outside of the uterus. Severe pelvic pain is often associated with endometriosis, and this pain can be diminished with therapies that suppress estrogen production. Many women with endometriosis also suffer from other chronic pain conditions. Recent studies suggest that mechanisms underlying these pains and sensitivity to estrogen involve the growth into the ectopic endometrial tissue of a nerve supply, which could have a varied and widespread influence on the activity of neurons throughout the central nervous system.

Endometriosis is a common disorder that occurs mainly in women of reproductive age. Because ectopic endometrial implants respond to natural or induced decreases in estrogen levels, the disorder is considered "estrogen dependent" (*1*). Symptoms of endometriosis include reduced fertility and several types of pain such as severe dysmenorrhea (excessive menstrual pain), deep dyspareunia (pelvic pain with coitus), dyschezia (pelvic pain with defecation), and chronic pelvic pain. In some women, pain can be exacerbated by the co-occurrence of other severe chronic pain conditions such as irritable bowel syndrome, interstitial cystitis, repetitive kidney stones, vulvodynia, temporomandibular syndrome, migraine, and fibromyalgia (*2*–*4*). Little is known about the association between the ectopic implants and pain; however, recent studies of women and animal models are beginning to provide clues.

The most common pharmacological treatment for endometriosis uses a class of drugs called gonadotropin-releasing hormone (GnRH) agonists. Because these drugs down-regulate GnRH receptors, they suppress pituitary gonadotropin secretion and sex steroid production, thereby producing a systemic hypoestrogenic state. This treatment results in the elimination or reduction in size of the implants in women (*5*) as well as in a rat model of endometriosis (*6*). In addition, optimized treatment with GnRH agonists is effective in reducing endometriosis-related pain symptoms in women (*5*).

Because GnRH agonists reduce both implant size and the pains associated with endometriosis, these pains may be due to the presence of the abnormal implants. Numerous studies, however, have failed to find a correlation among pain scores, types of pain, and various aspects of the anatomy and biochemistry of the implants (*7*). In addition, although surgical removal of the ectopic implants alleviates pain symptoms in many women, the surgery can fail to alleviate the pain and/or pain may recur even without evidence of residual or recurrent disease or any other identifiable visceral or somatic pathology (*8*).

On the other hand, correlations have been found between pain severity and both the depth of "infiltration" into peritoneum or pelvic organs and the proinflammatory cytokines, pros-

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taglandins, chemokines, and other substances released by the implants or neighboring tissues into peritoneal fluid (*1*, *9*). Of relevance here are findings that the percentage of patients reporting pain is greater in women with deeply infiltrating implants in highly innervated areas (such as the uterosacral region) than in women with other types of implants, and that the former implants are more likely to infiltrate nerves (*10*). Others have found that the nerve fibers are closer to the implants in women with pelvic pain than in women without pain (*11*). These results implicate the nervous system in the various pains of endometriosis. Indeed, recent results drawn from a rat model of endometriosis support this idea. This model (Fig. 1A) involves autotransplantation of parts removed from one uterine horn onto abdominal blood vessels, where the transplants then grow into cysts.

This rat model appears to be valid for studying both the signs of endometriosis (ectopic implants) and its symptoms (subfertility and pain). The implants in rats and women respond similarly to hormonal treatment and show similar alterations in protein production (*12*). Rats with endometriosis (ENDO rats), like some women with endometriosis, are subfertile (*12*). Although ENDO rats do not exhibit spontaneous pain behaviors (*13*), they develop an increased pain sensitivity (hyperalgesia) of the vagina, the severity of which correlates positively with estradiol levels during their ovarian cycle (*14*). Thus, ENDO rats suffer from a painful condition that is estrogen sensitive and is similar to dyspareunia in women. Furthermore, urinary bladder capacity is reduced in ENDO rats (*14*). In interstitial cystitis in women, the most salient symptom, other than pain, is excessively frequent urination. Endometriosis in rats also exacerbates pain behaviors associated with an artificial stone implanted in the ureter, and the stone in turn induces new pain behaviors like those that occur when the uterus is treated with an inflammatory agent (*13*). Thus, ENDO rats appear to develop pain symptoms similar to those associated with conditions that co-occur with endometriosis in women, specifically interstitial cystitis, uterine pain, and kidney stones (*4*).

The ectopic implants develop a sensory and sympathetic nerve supply both in rats (Fig. 1, B and D to F) and in women (Fig. 1, C and G to I), similar to that of the healthy rat uterus (*15*). In the rat model, this supply connects the implants directly with the central nervous system via the splanchnic and vagus nerves (*14*, *15*). Input to the spinal cord from the implants arrives at the same spinal segments as those receiving input from the ureter (Fig. 1A, green shading), but rostral to segments receiving input from the vaginal canal (blue shading) or bladder (pink and blue shading). Thus, vaginal hyperalgesia and ureteral pain in this rat model likely involve central neural mechanisms [i.e., intersegmental spinal communication (*14*, *15*)], whereas the effects of endometriosis on bladder function could be via peripheral interactions and/or in the caudal spinal cord.

Two other factors may help to explain the variable types and severity of endometriosis-associated pains, their co-occurrence with other painful disorders, and their amelioration by a hypoestrogenic state: (i) central sensitization, and (ii) divergent and convergent connectivity in the central nervous system. First, it is commonly recognized that sensory fibers of the type observed in both the rat and human ectopic implants (Fig. 1, F and I, respectively) are activated and sensitized by many inflammatory agents in them (*1*, *9*, *16*). By means of several molecular processes reviewed else-

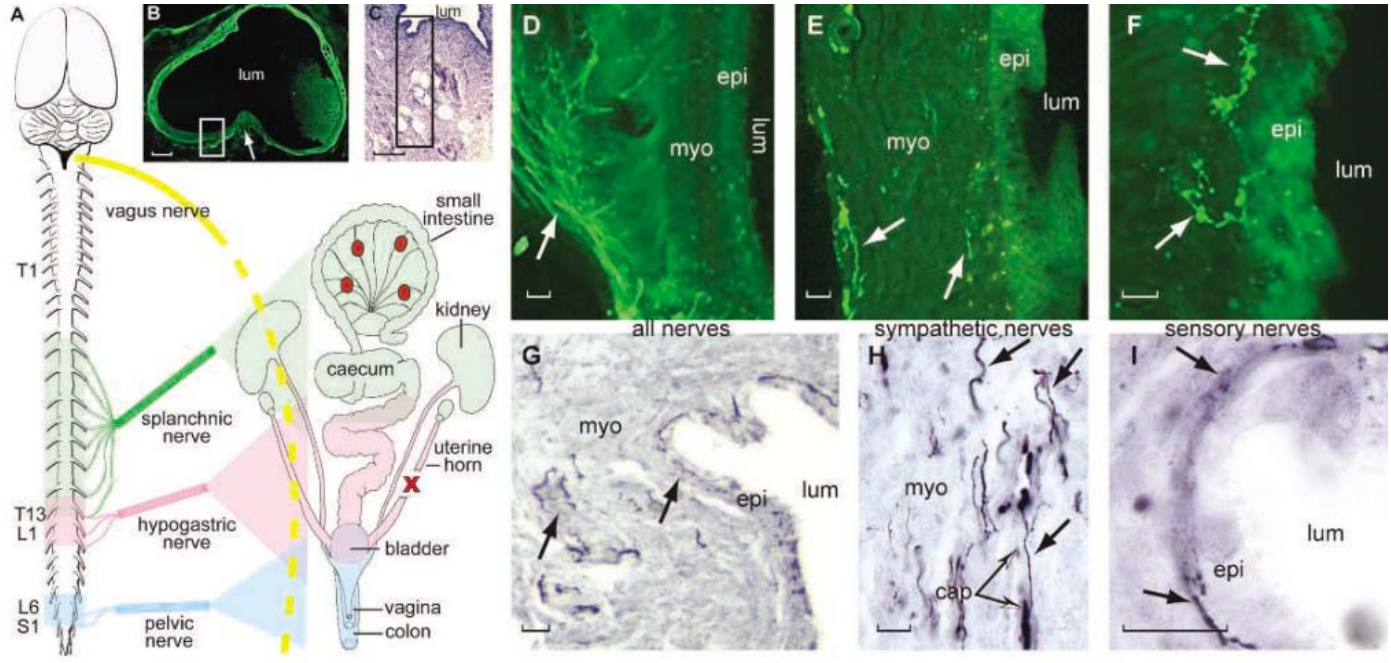

Fig. 1. (**A**) Rat model of endometriosis. Abdominal and pelvic organs are on the right; at left is the pattern of their input/output neural connections with different segments of the spinal cord and brain (via the vagus nerve). T1, T13, L1, L6, and S1 designate spinal segments. Also shown is the location of ectopic endometrial cysts (red dots) that develop after autotransplantation of pieces of uterus taken from one uterine horn (X) in the rat model of endometriosis discussed in the text (*12*–*15*). (**B**) Section through a rat endometrial cyst and lumen (lum) showing immunofluorescence for the pan-neuronal marker PGP9.5 (protein gene product 9.5); note bundle of nerves (arrow) entering the hilus. The box indicates an area from which images (D) to (F) were taken. (**C**) Section through a human cyst and lumen stained with hematoxylin and eosin. The box indicates an area from which images (G) to (I) were taken. (**D**) Hilus with nerves entering the cyst (arrow) and coursing toward the luminal epithelium (epi). (**E**) Hilus immunostained with markers for efferent nerves [vesicular monoamine transporter (VMAT), sympathetic fibers] in rat cysts. (**F**) Hilus immunostained with markers for sensory nerves [calcitonin gene–related peptide (CGRP)] in rat cysts. Neurites (arrows) are evident in the myometrial stroma (myo) and near the luminal epithelium. In (F), sensory fibers (upper arrow) enter the luminal epithelium. (**G** to **I**) Sections through human cysts were also immunostained for PGP9.5 (G), VMAT (H), and CGRP (I). (G) PGP9.5 fibers (arrows) in myometrial stroma and near the epithelium. (H) VMAT-sympathetic fibers (arrows) in myometrial stroma (myo) and along a capillary (cap; thin arrows). (I) CGRP-sensory fibers near and in the luminal epithelium. Scale bars, 250 μm (B), 100 μm (C), 25 μm [(D) to (I)]. [(A) modified from (*14*); (B), (D), (E), and (F) modified from (*15*)]

where (*16*), sensitization of the sensory fibers would in turn produce central sensitization, which is a long-lasting hyperexcitability of neurons in the central nervous system that can continue long after the originally sensitized input is reduced or eliminated (e.g., by surgery).

Second, sensory input arriving at the spinal cord from individual internal organs diverges within the cord. Thus, although information from different organs is delivered most densely to spinal neurons within the entry segments, it is also delivered, less densely, to widespread spinal regions extending for many segments rostrally and caudally (*14*). The anatomical divergence gives rise to considerable convergence of information on central neurons. This "viscero-viscero-somatic convergence" (*13*, *14*) produces a situation in which the activity of somato-visceral neurons in the spinal cord and brain is dominated by information from individual peripheral structures but can be augmented, particularly in sensitized neurons, by events occurring elsewhere. Such convergence thereby provides a substrate by which sensitized input from ectopic implants augmenting that from healthy organs can have widespread influences on the activity of neurons normally associated with input from different individual organs and tissues, a situation that has been demonstrated in women (*17*).

These results suggest that inconsistency in the various pains associated with the ectopic implants could reflect variability in a number of factors associated with the implants' nerve supply. These factors include the types of nerves that innervate the implants, agents that activate or sensitize them, sites in the central nervous system where the nerves deliver information, and how that information is modulated by estradiol—both peripherally (*18*) and centrally (*19*)—as well as by other central dynamic processes (*14*, *17*, *20*).

Much remains to be learned about how endometriosis comes to be associated so variably with pain symptoms and how those symptoms are ameliorated by a hypoestrogenic state. One promising area of research concerns the implants' sensory and autonomic nerve supply and its potentially estradiol-modulated influence on activity within the central nervous system.

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REVIEW

Uterine Fibroids: The Elephant in the Room

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Uterine fibroids (leiomyomas) have historically been viewed as important chiefly as the major indication for hysterectomy. As new therapies are developed, the heterogeneity of this disease becomes therapeutically relevant. An awareness of the role of genetics, the extracellular matrix, and hormones in tumor etiology is key to understanding this disease.

Uterine fibroids (correctly called leiomyomas or myomas) are benign myometrial neoplasms enriched in extracellular matrix (ECM) (Fig. 1) (*1*). They are the primary indication for hysterectomy, accounting for over 200,000 hysterectomies annually in the United States, and are the cause of significant morbidity from profuse menstrual bleeding and pelvic discomfort. The range of clinical disease is extraordinary: Symptomatic lesions can be 10 mm in size or routinely exceed 20 cm. Tumors occur in 77% of women, and approximately 25% of Caucasians have clinically significant lesions (*2*). However, in African-American women, clinical disease is more severe and concordant with prevalence.

Rarer lesions such as the poor-prognosis leiomyosarcoma are providing insights into the biology of these benign tumors. Although it has been debated whether leiomyomas and leiomyosarcomas are part of a disease continuum, cytogenetic studies have demonstrated that chromosome rearrangements in leiomyomas are similar to those seen in other benign tumors but are distinct from the complex rearrangements and aneuploid karyotypes characteristic of leiomyosarcomas (*3*). However, recent microarray data from the Morton laboratory identified a rare subset of leiomyomas with deletions of chromosome 1 that have transcriptional profiles that cluster with those of leiomyosarcomas (*4*), suggesting that some uterine leiomyosarcomas may in fact arise from a specific subset of leiomyomas. There are also related lesions with both benign and malignant features. Benign metastasizing uterine leiomyoma is characterized by leiomyoma-like lesions, usually in the lungs, in women with fibroids. Lymphangioleiomyomatosis is a similar disease, affecting only women, in which the characteristic lung lesions originate from "benign" renal angiomyolipomas (*5*). Similarly, intravenous leiomyomatosis (IVL) is a hormonally responsive disease that causes vermiform extensions originating in the uterus that can extend as far as the heart. These leiomyomas have cytogenetic alterations similar to those seen in atypical lipomatous tumors that are locally invasive but do not metastasize (*6*).

Risk Factors and Prevalence

The prevalence of clinically significant fibroids peaks in the perimenopausal years and declines after menopause (*7*). Obesity and early age at menarche, which increase a woman's overall lifetime exposure to estrogen, are known risk factors. The risk of developing fibroids is higher in African-American than in Caucasian women, and they often have more severe disease. Parity is also a significant risk factor, with age at the birth of the last child being inversely associated with risk, suggesting that pregnancy may remove nascent tumors or promote regression, as is thought to be the case with endometrial cancer. Pregnancy reveals the extraordinary extent to which myometrial smooth muscle cells (MSMCs) can grow without malignant transformation. One hypothesis, proposed by Barbieri and Andersen, is that

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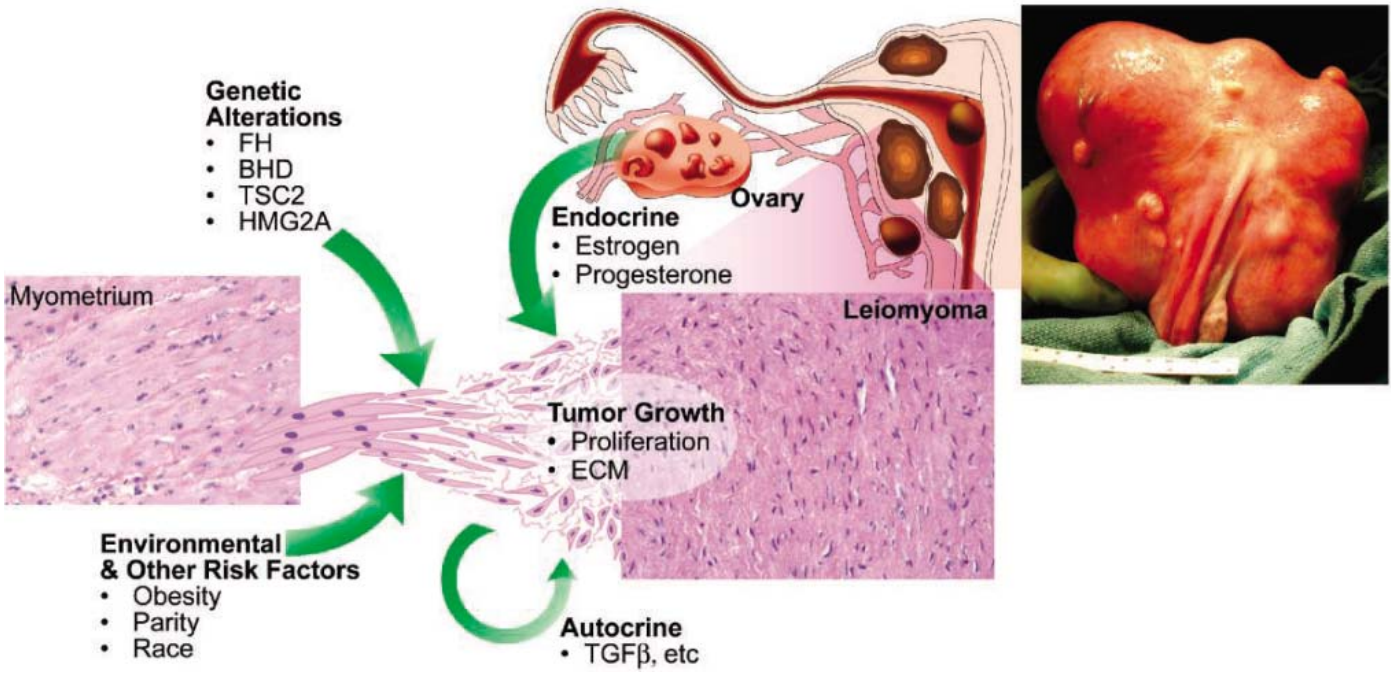

Fig. 1. Etiology of uterine fibroids. Leiomyomas are heterogenous in their natural history and etiology. Hereditary defects in the *FH*, *BHD*, and *TSC2* genes and somatic alterations affecting *HMG2A* genes contribute to the development of fibroids, as do risk factors such as obesity, parity, and race. Tumor growth occurs by an increase in tumor cell number and ECM production and is promoted by both endocrine and autocrine growth factors.

leiomyoma cells have assumed the phenotype of MSMCs of pregnancy (*8*). Indeed, leiomyomas share many characteristics with the parturient myometrium, including increased production of ECM components; the expression of receptors for peptide and steroid hormones; and expression of the gap junction protein connexin 43, which is required for cell-cell communication and the synchronous contractions of labor. However, leiomyomas fail to regress via apoptosis and the dedifferentiation that is characteristic of the postpartum myometrium, potentially because of differences in the expression of COX-2 and prostaglandins, the final mediators of parturition (*9*). The protective effect of pregnancy, which would induce these mediators, is consistent with this hypothesis.

Genetics of Uterine Leiomyoma

Uterine leiomyomas were the first tumor for which glucose-6-phosphate dehydrogenase (G6PD) was used to establish that these tumors are clonal in origin (*10*). Although the majority of uterine leiomyomas are cytogenetically normal, approximately 40% display nonrandom cytogenetic alterations, frequently involving chromosome 12 (*11*, *12*). Translocations involving this chromosome identified the target as the *HMGA2* (formerly *HMGI-C*) gene, a member of the high-mobility–group gene family of DNA architectural factors. HMGA2 and HMGA1 (formerly HMGI-Y) are both frequently aberrantly expressed in fibroids and other benign mesenchymal lesions, including lipomas.

Several hereditary cancer syndromes predisposing to leiomyomas suggest a genetic linkage with renal cell carcinoma (RCC) (*13*). These syndromes include hereditary leiomyomatosis and RCC (HLRCC), tuberous sclerosis complex (TSC), and Birt-Hogg-Dubé (BHD) syndromes. For example, individuals with HLRCC, who have mutations in the fumarate hydratase (*FH*) gene, develop papillary RCC and uterine and skin leiomyomas. Furthermore, Eker rats that carry a germline defect in the rat homolog of the *Tsc2* gene develop spontaneous RCC and uterine leiomyomas with a high frequency (*14*). TSC patients develop renal angiomyolipomas and are at increased risk for RCC, and many sporadic human leiomyomas exhibit loss of function of the *TSC2* gene product tuberin (*15*). Benign cutaneous lesions and uterine leiomyomas also arise in German shepherd dogs with germline mutations in the *Bhd* gene that develop RCC. Although the biological basis for this apparent shared genetic etiology is not clear, the fact that kidney epithelial cells share a mesenchymal lineage with myometrial cells may be an important clue (*16*).

Unfortunately, the clinical relevance of these genetic syndromes is not widely appreciated. The finding of cutaneous leiomyomas (as seen in HLRCC) should prompt screening of the family not only for RCC but for uterine sarcomas, which are more frequent in these patients. The fact that genetic heterogeneity underlies the dramatic clinical heterogeneity of leiomyomas is also becoming clear. For example, although FH alterations in nonsyndromic leiomyomas are rare, in a cohort of patients we examined, FH alterations were seen in leiomyomas of Caucasian but not African-American women (*17*). This observation underscores the importance of genome-wide scanning in a racially diverse population, such as the Finding Genes for Fibroids Project (www.fibroids.net) at Brigham and Women's Hospital in Boston.

Hormones and Growth Factors in Pathogenesis

Like most reproductive tract tumors, fibroids are steroid hormone–dependent (*18*). Leiomyomas are hyperresponsive to estrogen and exhibit elevated levels of estrogen and progesterone receptors (ERs and PRs), with the expression of a dominant-negative ER inhibiting leiomyoma cell growth in vitro and in vivo (*19*). Leiomyomas also exhibit alterations in estrogen metabolism, including elevated aromatase levels (*20*). Although estrogen has traditionally been identified as the sole pathogenic influence, an alternative hypothesis posits that progesterone is the dominant steroidal influence. The "progesterone hypothesis" (*21*) is supported by increased mitotic rates in myomas during the secretory phase, when progesterone levels peak (*22*), and by clinical data indicating that progestins inhibit the therapeutic effect of GnRH agonists. The utility of mifepristone (RU486) in treating fibroids also supports this hypothesis. However, transdominant suppression of ER signaling by PR ligands has been demonstrated, with both progestins and antiproges-

tins capable of suppressing ER signaling and leiomyoma cell growth (*18*). These data suggest that cross-talk between ER and PR signaling occurs in uterine leiomyomas and that the effects of steroidal ligands may be mediated by disruption of ER, PR, or even androgen receptor signaling.

Many cytokines and growth factors may also foster leiomyoma growth through paracrine and/or autocrine mechanisms. These include transforming growth factor–β (TGF-β), insulin-like growth factors 1 and 2 (IGF-1/2), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) (*23*). In some instances, growth factor expression is modulated by steroids, suggesting that they are the ultimate effectors of steroid hormone action.

Given the low mitotic index of leiomyomas, growth factors probably contribute to myoma enlargement by stimulating the deposition of ECM. TGF-β in particular is thought to be a central player in ECM production (*24*). Leiomyoma cells express TGF-β receptors and SMADs and overexpress TGF-β relative to normal myometrium. Downstream targets of TGF-β signaling, such as tissue inhibitor of matrix metalloproteases and plasminogen activator inhibitor, which promote ECM production, are also overexpressed in myomas. Recently, transcriptional profiling identified a number of TGF-β–responsive genes overexpressed in leiomyoma cells, including interleukin-11, which plays a major role in other fibrotic disorders (*25*). GnRH agonists inhibit TGF-β expression, and reduced expression of this cytokine may underlie ECM reduction and clinical shrinkage in tumors in response to GnRH therapy. Aberrant TGF-β signaling is associated with decreased dermatopontin expression, hinting at a molecular link between leiomyomas and keloids (scar tissue), which are both more prevalent in African-American women (*26*). Recent work also suggests that hypoxia may participate in the development of myomas in HLRCC patients (*13*), lending support to the theory that fibroids form as a response to injury (*27*).

Growth factors and ECM also contribute to the profuse menstrual bleeding seen in women with leiomyomas (*28*). Besides stimulating the production of matrix components that promote angiogenesis, several of the growth factors expressed by fibroids are themselves vasoactive. These include bFGF, which promotes angiogenesis; parathyroid hormone–related protein, a potent vasorelaxant that reduces vascular tone; and prolactin, which can act either as a proangiogenic factor by inducing vascular endothelial growth factor or as an antiangiogenic factor when cleaved by cathepsin D. The abundant ECM also serves as a reservoir for many angiogenic heparin-binding growth factors such as bFGF and heparin-binding epidermal growth factor, as well as PDGF and prolactin. Both mifepristone and onapristone have been shown to suppress prolactin production by leiomyomas in vitro, which may be linked to the clinical efficacy of progesterone-modulating therapy.

Model Systems

Several animal models for uterine leiomyoma exist. The best-characterized and most widely used is the previously mentioned Eker rat model (*14*), in which approximately 65% of female $Tsc2^{Ek/+}$carriers develop leiomyomas, a frequency similar to that seen in women. Eker rat leiomyomas share phenotypic, biochemical, and genetic characteristics with the cognate human disease, including ER and PR expression and responsiveness to steroid hormones, aberrant HMGA2 expression, overexpression of IGF-1, and the protective effects of pregnancy. Recently, this model was used to demonstrate that brief exposure to a xenoestrogen during the development of the myometrium can reprogram the response of this tissue to estrogen and promote leiomyoma development in the adult (*29*). Cell lines have been established from these tumors, which, unlike human leiomyoma cells, retain their hormone responsiveness in vitro. This in vivo/in vitro model system has demonstrated the potential efficacy of selective ER modulators (SERMs), PR modulators (SPRMs), and drugs that target peroxisome proliferator-activator receptors (PPARs) as therapeutic agents for fibroids (*18*).

A guinea pig model also exists. Approximately 8% of aged guinea pigs develop spontaneous leiomyomas, and ovariectomy in young animals combined with high-dose estrogen supplementation causes the development of uterine and abdominal leiomyomas with a high frequency. Preclinical studies in these animals also suggest that SERMs and PPAR ligands may be efficacious as therapeutic agents (*30*).

No mouse models for this disease are available, but a handful of studies have shown that the β-adrenoceptor antagonist levobunolol induces leiomyomas in mice and that transgenic mice expressing SV40 T antigen driven by the estrogen-responsive calbindin promoter develop leiomyomas (*31*, *32*). Human myometrial and leiomyoma cells have now been immortalized through the expression of telomerase (*33*, *34*), and these cell lines hold promise for future development of preclinical screening assays.

Therapy: Much Room for Improvement

Historically, there has been little innovation in treatments for fibroids, nominally because they are benign and cause morbidity, not mortality, and because leiomyoma research is underfunded as compared with that for other nonmalignant diseases (fig. S1). The standard treatment of uterine fibroids—surgical excision and hysterectomy—has been promulgated as the one-size-fits-all solution. However, although the endoscopic resection of fibroids that are accessible from either surface of the uterus has been a surgical advance, the vast majority of fibroids lie within the uterine wall (are intramural) and are difficult to treat in a minimally invasive fashion.

In the past decade, uterine artery embolization (UAE) has provided an intramural leiomyoma treatment (*36*). Two embolic agents are approved for UAE, accounting for half of all U.S. Food and Drug Administration (FDA)–approved therapies for treating uterine fibroids. Nonetheless, the induction of global uterine ischemia is still a rather crude approach, and there are ongoing concerns regarding the impact of UAE on fertility and pregnancy.

Magnetic resonance imaging–guided focused ultrasound surgery (MRgFUS) is a therapy for leiomyoma treatment that received FDA approval in October 2004. Although this technique promises noninvasive thermoablative therapy, its chief importance currently is that it is the first technology approved for fibroid treatment as the primary indication. The indications for this therapy are likely to expand, because MRgFUS is able to target specific leiomyomas and provide for outpatient therapy (*37*).

Medical therapy for fibroids is similarly limited. The only FDA-approved medical therapy is a GnRH agonist used preoperatively with iron. GnRH agonists abrogate both bleeding and bulk-related symptoms but induce significant menopausal side effects that limit therapy. Tumors also rapidly regrow if not removed surgically. Consequently, there is great interest in developing drugs to treat myomas. Unlike their success in preclinical studies, to date SERMs have yielded disappointing results in clinical trials. Current research is investigating progesterone modulation achieved with both traditional drugs such as mifepristone and newer SPRMs such as asoprisnil. Smaller studies are also seeking new avenues for fibroid treatments, including antifibrotic agents and growth factor antagonists.

Conclusion

The fact that fibroids are nonmalignant should not imply that they are benign in their impact on women's health. Although gonadal steroids play an important role in their pathogenesis, multiple other molecular targets for potential therapeutic innovation have been elucidated. The lack of discourse on all menstrual disorders, and particularly uterine fibroids, has placed affected women and interested scientists at a tremendous disadvantage. There is ample room for additional biomedical research and therapeutic innovation for dealing with this important disease.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/308/5728/1589/DC1

Fig. S1

10.1126/science.1112063

REVIEW

Latest Advances in Understanding Preeclampsia

Christopher W. Redman* and Ian L. Sargent

Preeclampsia is a relatively common pregnancy disorder that originates in the placenta and causes variable maternal and fetal problems. In the worst cases, it may threaten the survival of both mother and baby. We summarize recent work on the causes of preeclampsia, which reveals a new mode of maternal immune recognition of the fetus, relevant to the condition. The circulating factors derived from the placenta, which contributes to the clinical syndrome, are now better understood. This brief review on preeclampsia does not cover all aspects of this intriguing condition but focuses on some new and interesting findings.

Preeclampsia is a potentially dangerous complication of the second half of pregnancy, labor, or early period after delivery, characterized by hypertension, abnormal amounts of protein in the urine, and other systemic disturbances (*1*). The condition affects about 2.5 to 3.0% of women. It has the potential to kill either mother or baby or both, even in the developed world (although rarely). Eclampsia is an end stage of the disease characterized by generalized seizures. Preeclampsia cannot be prevented, so it is managed by screening symptomless women and inducing delivery when necessary. It is one of the most common reasons for induced preterm delivery.

Risk factors for preeclampsia have been analyzed in a recent systematic review (*2*). These factors include a previous history of preeclampsia, primiparity, obesity, family history of preeclampsia, multiple pregnancies, and chronic medical conditions such as long-term hypertension or diabetes (*2*). Paradoxically, cigarette smoking reduces the risk (*2*). Thrombophilia, an inherited tendency to overactive coagulation, may also be a consideration. Although preeclampsia may develop at any time after 20 weeks of gestation, early onset disease is more severe and characterized by a higher rate of small size for gestational age neonates as well as a higher recurrence rate than with later onset disease.

It is generally agreed that preeclampsia results from the presence of a placenta (*3*) and, in particular, the trophoblast cells that are found only in this tissue. Multinucleate syncytiotrophoblast, which forms the epithelial layer of the villi, is one subset of trophoblast that is in direct contact with maternal blood. Mononuclear extravillous cytotrophoblast form a tissue interface in the lining of the uterus, the decidua. The clinical syndrome arises from secondary systemic circulatory disturbances that can be ascribed to generalized maternal endothelial dysfunction. There are two broad categories, maternal and placental. In placental preeclampsia, the problem arises from a placenta that is under hypoxic conditions with oxidative stress. (*4*) Maternal preeclampsia arises from the interaction between a normal placenta and a maternal constitution that is susceptible to, or suffers from, microvascular disease, as with long-term hypertension or diabetes (*5*). Mixed presentations, combining maternal and placental contributions, are common.

Placental Preeclampsia

Placental preeclampsia appears to progress in two stages: preclinical and clinical (Fig. 1C). This variant arises from poor development of the early placenta and its maternal blood supply, called poor placentation. In the second stage, an increasingly hypoxic placenta causes the maternal signs of the condition, including hypertension and proteinuria as well as clotting and liver dysfunction. In severe, particularly early onset disease (before 34 weeks gestation), the fetus may suffer increasing nutritional and respiratory insufficiency, asphyxia, or death.

In the second two trimesters of pregnancy, the placenta requires increasing access to the maternal blood supply. This is created by extensive remodeling of maternal spiral arteries, which are the end arteries of the uteroplacental circulation that deliver blood directly into the placental intervillous space. Remodeling depends on one of the subtypes of the trophoblasts, which differentiates into tumor-like cells (extravillous cytotrophoblasts) that invade the lining of the pregnant uterus from weeks 6 to 18 of gestation (*6*).

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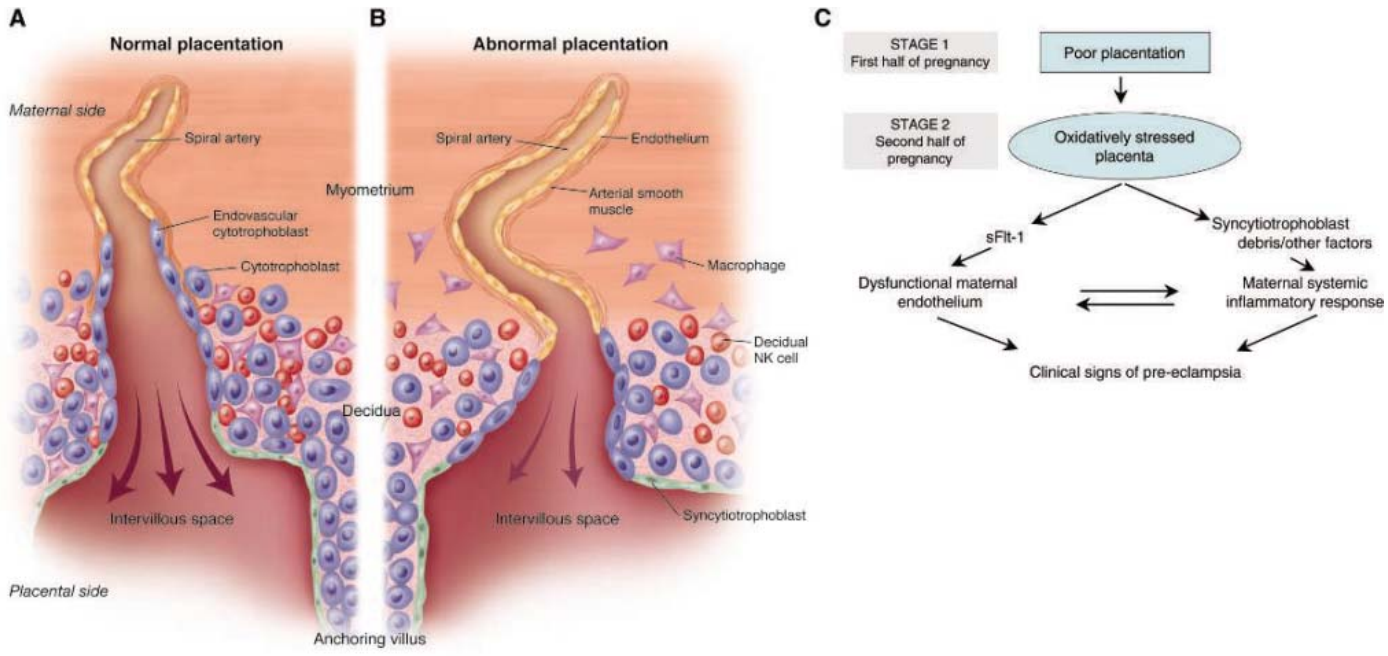

Fig. 1. Poor placentation and preeclampsia. Normal placentation (**A**) and poor placentation (**B**) at 15 to 16 weeks of pregnancy. The placenta is linked to the maternal decidua by anchoring villi. During normal placentation, cytotrophoblasts (blue) cross these placental-maternal bridges and invade the maternal decidua and adjacent spiral arteries. They penetrate the walls of the arteries and replace part of the maternal endothelium (yellow), stimulating remodeling of the arterial wall such that the smooth muscle is lost and the artery dilates. In the decidua, they are confronted by many NK cells (red) and some macrophages (purple). During normal pregnancy, these immune cells facilitate deep invasion of cytotrophoblasts into the myometrial segments (A) and promote extensive spiral artery remodeling. In the preclinical stage of preeclampsia, invasion is restricted (B) with impaired arterial remodeling. (**C**) The two stages in development of preeclampsia.

The trophoblasts penetrate the maternal decidual spiral arteries, which are obstructed before 9 weeks of gestation by invasive trophoblast plugs (*4*, *7*) when placental perfusion is minimal. Before this time, the fetus is engaged in organogenesis and is especially vulnerable to teratogenic damage from free radicals. After 9 weeks, the uteroplacental arteries recanalize from the placental periphery, a process that is completed by 12 weeks. The associated increase in placental oxygenation is a watershed for trophoblast growth and differentiation and marked by the sudden appearance of markers of oxidative stress in the placenta (*7*). From then on, invasive cytotrophoblasts in decidual tissue extensively remodel the spiral arteries, including their distal myometrial segments, such that they lose their smooth muscle and become greatly dilated (Fig. 1). The presence of trophoblasts in the lumina (endovascular cytotrophoblasts), walls, and surrounding interstitial tissues (interstitial cytotrophoblasts) of the ends of the spiral arteries appears to be critical for this process. Endovascular trophoblasts express markers of endothelial cells, including angiogenic factors and their receptors, and replace the endothelial lining of the arteries with a pseudoendothelium forming intriguing compound vessels, part fetal and part maternal (*6*).

By 20 weeks, this process is more or less complete such that the maternal circulation can supply the expanding intervillous space of the placenta; by full term, a huge fetal surface formed from the microvilli (the terminal leaflets of the branching umbilical circulation, covered in the syncytiotrophoblasts) can come into direct contact with maternal blood. Hence, extravillous cytotrophoblasts play a critical role by expanding the vascular capacity of the uteroplacental circulation. In many cases of placental preeclampsia, trophoblast invasion is inhibited, the arteries are poorly remodeled, and the capacity of the uteroplacental circulation is too small. This is called poor placentation, which is established before 20 weeks and before clinical signs appear (*3*). It is impossible to study this process prospectively, but it has been inferred from studies of placental bed biopsies at delivery. In addition, poor placentation does not always cause overt preeclampsia but has been associated with small size for gestational age fetuses.

Immunological Considerations

In preeclampsia, invasive trophoblasts fail to gain full access to maternal supply lines. New work suggests that trophoblast signaling to decidual immune cells is weak and fails to stimulate collaboration, essential for placentation (*8*, *9*). It has long been considered that preeclampsia may be a form of maternal immune rejection of the genetically foreign fetus. Many have sought maternal T cell recognition of fetopaternal human lymphocyte antigens (HLAs) without success, because trophoblasts do not express the necessary strong transplantation antigens, HLA-A, -B, or -D (*10*). Invasive cytotrophoblasts that infiltrate maternal territory during placentation express a unique combination of HLAs, namely HLA-C, -E, and -G (*10*). Of these, only HLA-C is polymorphic signaling paternal (foreign) alloantigens. In the decidua, the invading trophoblasts meet many maternal lymphocytes. These are not classical T cells but mainly NK (natural killer) cells with an unusual phenotype when compared to circulating NK cells (10), which is associated with high cytokine production rather than cytolytic activity. Of outstanding importance is the fact they express receptors that recognize the exact combination of HLAs displayed by invasive cytotrophoblasts. The invasive cytotrophoblasts and the decidual NK cells are closely apposed to each other in the first trimester decidua but disappear by full term (*11*). Because the NK cells express an unusual array of receptors that bind to the unique combination of HLAs expressed by the intermingling cytotrophoblast and NK cells, it is likely they engage in a some form of immune recognition (*10*).

The NK receptors KIRs (killer immunoglobulin-like receptors) recognize polymorphic HLA-C. The multigene KIR generates numerous haplotypes that differ in both gene content and allele combination. Some haplotypes inhibit NK cell function (cytokine production in these cells), whereas others are stimulatory, depending on both the KIR pheno-

type of the NK cells and the HLA-C phenotype of the stimulating cells. There are a large number of possible combinations. However, there are two broad classes of HLA-C haplotypes (C1 and C2). The KIRs bind more strongly to C2 than C1. KIR haplotypes also form two groups. The simpler A group codes mainly for inhibitory KIR. The more complex B group has additional genes for stimulating NK cells (*9*). Preeclampsia is substantially more prevalent in women who are homozygous for the inhibitory A haplotypes (AA) than in women homozygous for the stimulator B genes (BB). The effect is strongest if the fetus is homozygous for the HLA-C2 haplotype (9). In short, placentation is better and preeclampsia less common if trophoblast strongly simulates uterine (maternal) NK cells. However, this activation has not yet been confirmed in vitro. Whether this interaction between trophoblasts and NK cells can help to explain why preeclampsia is more common in first pregnancies, long suggested to be an immune phenomenon, remains to be seen.

Placental Factors That Might Cause the Maternal Syndrome

In the two-stage model, a hypoxic and dysfunctional placenta is considered to release factors into the maternal circulation that cause the clinical features of this condition (Fig. 1C). These appear to arise from a generalized systemic inflammatory response (*12*), of which endothelial dysfunction is a prominent component. Several candidate factors have been suggested (*12*); none of them are yet proved to be causative in vivo. The strongest is the soluble receptor for vascular endothelial growth factor (VEGF)–1, also known as sFlt1 (soluble fms-like tyrosine kinase 1). It binds vascular endothelial growth factors and placental growth factor and deprives the systemic endothelium of essential survival factors. It is therefore antiangiogenic, as has been confirmed in animal and human studies [summarized in (*12*)].

Infused neutralizing monoclonal antibody to VEGF mimics the anti-angiogenic action of sFlt1, which has been exploited to treat metastatic colorectal or renal cancer. It also causes hypertension and proteinuria, the typical features of preeclampsia [summarized in (*13*)]. It has been recently reported that preeclamptic women have higher circulating sFlt1 concentrations than do normal pregnant control women and also more anti-angiogenic activity in their sera. Moreover, infusion of sFlt into experimental rats elicits endothelial lesions in the glomeruli of the kidneys (glomerular endotheliosis) that are pathognomic of preeclampsia (*13*). sFlt1 is not specific to pregnancy, but the factor is secreted into maternal blood by trophoblasts, which are stimulated by hypoxia. It therefore has the necessary attributes of a trophoblast-derived factor that disrupts the maternal endothelium, which is a primary target for preeclampsia (*1*). However, it may not be the sole cause, nor is it raised in every affected woman. The term "preeclampsia" describes a syndrome (a cluster of clinical features) not a disease and may encompass separate conditions that look alike to the clinician. The condition is varied in its presentation, features, and outcomes. It is hard to conceive how one factor can explain the entire spectrum. Another suggested candidate is neurokinin B (*14*). There are also many other circulating trophoblast factors that are increased in preeclampsia, and their role in the disease is undefined.

Preeclampsia is associated with a systemic inflammatory response (*15*), but so is normal pregnancy, although to a lesser extent. Many parts of the inflammatory network are involved (inflammatory leukocytes, endothelium, clotting cascade, platelets, and acute phase reactants) yielding minor systemic changes that have previously been considered to be part of the physiology of normal pregnancy. These features are intensified in the third trimester, even more so in preeclampsia, and could contribute to some, if not all, of its maternal disturbances. The causes of the systemic inflammation are not known. One intriguing feature is that the placenta releases what can be described as trophoblast debris into the maternal circulation. This comprises syncytiotrophoblast membrane microparticles, cytokeratin fragments, soluble RNA and DNA of fetal origin, and even cytotrophoblast cells (*15*). This debris is proinflammatory and increased in amounts in preeclampsia, so its disposal probably imposes a maternal inflammatory burden.

The hypoxic placenta of preeclampsia suffers oxidative stress, a disequilibrium between antioxidant defenses and production of reactive oxygen species in favor of the latter. Its markers are readily detected in the preeclampsia placenta. Such stress is probably the cause of the increased release of trophoblast debris by apoptotic processes, exacerbated further by necrosis (*16*), although this is not yet proved. This, and the evidence for systemic oxidative stress in preeclamptic women, has provoked an encouraging trial of antioxidant vitamins C and E for prophylaxis. This showed substantial alleviation of the maternal signs but not an improved perinatal outcome. Larger confirmatory trials are underway in several countries. If the initial benefit is confirmed, this could be a major advance in preventive management (*17*).

Maternal Preeclampsia

A similar low-grade systemic inflammatory response characterizes adults with arterial disease, hypertension, obesity, or diabetes, which are conditions that also strongly predispose to preeclampsia in young women. Such constitutions lead to "maternal preeclampsia," where the problem is more an abnormal maternal response than an abnormal pregnancy (5). In this regard, pregnancy may constitute a metabolic and vascular stress test, which reveals a woman's health in later life and which is consistent with the higher incidence of ischemic heart disease, stroke, and hypertension that becomes evident many years after an episode of preeclampsia (*18*). Although preeclampsia is familial, it does not depend on a single maternal or fetal gene. There are many candidate genes that cannot be described here but are reviewed elsewhere together with evidence for a new gene for a transcription factor expressed in the placenta that may be involved in the control of trophoblast invasion (*19*).

Survival of the Fittest?

In evolutionary terms, preeclampsia is perceived as a struggle between the different survival needs of maternal and paternal (fetal) genes or maternal-fetal conflict (*20*). It is suggested that preeclamptic hypertension is dictated by the fetus (placenta) to gain a greater share of the maternal circulation. The price in terms of risks of maternal death would seem to be evolutionarily acceptable.

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Observations by the International Tsunami Survey Team in Sri Lanka

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On 26 December 2004 at 00:58:53 universal time (U.T.), an earthquake of surface wave magnitude (M_s) 9.0 occurred off the west coast of northern Sumatra. Large tsunamis were generated that severely damaged coastal communities in countries around the Bay of Bengal and the Indian Ocean, including Indonesia, Thailand, Sri Lanka, and India. The estimated tsunami death toll ranges from 156,000 to 178,000 across 11 nations, with an additional 26,500 to 142,000 missing, most of them presumed dead.

A tsunami survey plan was initiated within 3 days of the earthquake; a survey team of eight scientists from the United States and one from New Zealand was formed and dispatched to Sri Lanka. The team was supported by four Sri Lankan scientists. The team surveyed both the east and southwest coasts of Sri Lanka during the period 10 January through 14 January 2005. The team measured maximum tsunami heights, maximum runup heights, inundation distances, and areas of inundation. We also collected soil samples from tsunami deposits, did a limited aerial inspection along the southwestern coast, and recorded eyewitness accounts.

The elevations of watermarks on buildings, scars on trees, and rafted debris were measured as indicators of the maximum tsunami height, which is defined relative to sea level. Inundation distance is the distance from the shoreline to the inland limit of tsunami flooding, and the maximum runup height is the elevation at the inundation distance. Every mark used for the measurement was photographed, and its location was identified using a global positioning satellite. Figure 1 shows the measured maximum tsunami heights and runup heights, adjusted for the tide levels at the time the tsunami hit.

Eyewitnesses described one to three waves, depending on the location, and provided estimates of their heights. From Matara at the southern tip to near Galle, the first wave arrived around 03:10 U.T. (9:10 a.m. local time) as a leading elevation wave (*1*) with a wave height less than 1 m, followed 10 min later by a second large elevation wave with wave height up to 10 m. The leading waves were refracted around the southern tip of the island and reached the west coast (*2*). North of Galle, up to Kaluthara, a third elevation wave with a height up to several meters was reported near noon, suggestive of reflection from the coast of India or from the Maldives. On the east coast, the first reported wave was an elevation that rose like a tide to ~1 m, followed by a depression, whereas the second elevation wave was large and fast. On both the east and southwest coasts, eyewitnesses reported a major recession of hundreds of meters in horizontal length between the first and second arrivals.

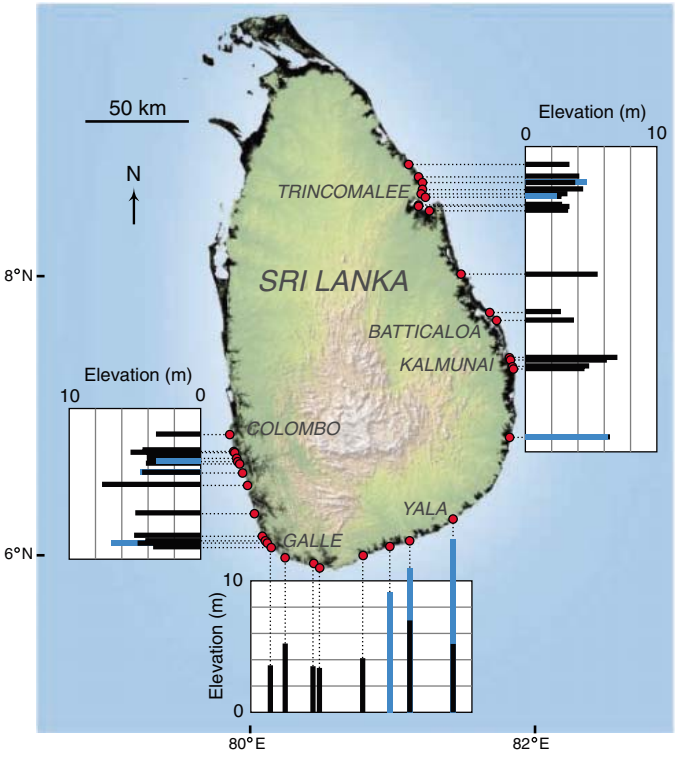

Fig. 1. Measured tsunami runups (blue) and maximum tsunami heights (black). Red dots show sites of elevation measurement; areas shaded in black are less than 10 m above sea level. The map is modified from one by NASA/GSFC/METI/ERSDAC/JAROS and ASTER.

The importance of tsunami education for coastal residents was exemplified in a small fishing village near Galle. One of the village residents, a merchant marine who witnessed a tsunami in Chile two decades ago, recognized the sea withdrawal as a tsunami indicator. He gave warning to run to higher ground, which nearly all his fellow residents followed. In this village of a few hundred, only one died.

We noted a number of instances where human development likely modified the runup behavior of the tsunami. The Sumudra Devi, a passenger train out of Colombo, was derailed and overturned by the tsunami, killing more than 1,000. In the immediate area, substantial coral mining had occurred, related to tourism development. Tsunami runup in the area was nearly 8 m. In the town of Yala, tourism activities also affected the tsunami runup. One resort, for the purpose of better scenic views, had removed some of the dune seaward of its hotel. The hotel was destroyed by the tsunami. Substantially larger water elevations and greater damage observations were found near the hotel, as compared to neighboring areas located behind unaltered dunes. In essence, by removing some of the natural coastal protection in a localized area, a conduit was created through which the tsunami energy could flow more freely.

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Field Data and Satellite Imagery of Tsunami Effects in Banda Aceh

Jose C. Borrero

The rapid response of researchers with the international tsunami survey team (*1*) to the Banda Aceh, Sumatra, region after the 26 December 2004 earthquake and tsunami led to the recovery of data on the characteristics of the tsunami inundation. These data were used in conjunction with satellite imagery to identify runup height (elevation above sea level), inundation distance, shoreline erosion, and coseismic subsidence. Working alone, the author collected field data consisting of cross-shore profiles and photographs (located by global positioning satellite) of watermarks suggestive of flow depths (elevation above ground level) and of flow directions. Wave arrival times and wave patterns were inferred through eyewitness interviews and video taken during the tsunami. Only a brief summary of the data is presented here.

Flow depths along the north-facing shoreline of Banda Aceh were in excess of 9 m and tapered landward to the extent of maximum inundation, 3 to 4 km inland. Eyewitnesses in Banda Aceh described a series of three waves, beginning with a leading depression *N* wave (one in which the trough reaches the shoreline first) (*2*). The second elevation wave was the most destructive. In Banda Aceh, the predominant flow direction was from the northwest (Fig. 1). Fifteen km to the southwest, at Lhoknga, bark stripped from trees suggested flow depths in excess of 15 m at the shoreline, with flows predominantly from the west. Runup heights here were estimated during the initial survey in excess of 25 m. This value was later confirmed by comparing the inundation line inferred in satellite images to digital elevation data on the coastal topography. Runup around a steep conical island, less than 50 m off the south end of Lhoknga, reached 31 m. The runup values around Lhoknga are consistent (within a factor of 1 or 2) with values for coseismic slip proposed for the faulting region offshore of this area. This supports Plafker's rule, which suggests that the maximum runup is never larger than twice the maximum seafloor displacement (*3*).

In the lowlands between Banda Aceh and Lhoknga, eyewitnesses reported waves from both the north and southwest. A comparison of before and after imagery allowed assessment of coastal erosion and subsidence and confirmed that two tsunami wave fronts merged across the northwestern tip of Sumatra. Alterations in the shoreline are clearly visible (Fig. 1). Standing water over what was previously dry land suggests subsidence. Wave scour and subsidence permanently moved the shoreline of Banda Aceh inland by as much as 1.5 km, and 65 km^2 of land between Banda Aceh and Lhoknga were flooded.

In this study, field data and remote sensing imagery were employed together to describe tsunami inundation. In future events, satellites could be directed to image affected regions and guide emergency response, allowing for more focused damage assessment and field measurements. The extent of the catastrophe underscores the need for real-time tsunami forecasting (*4*).

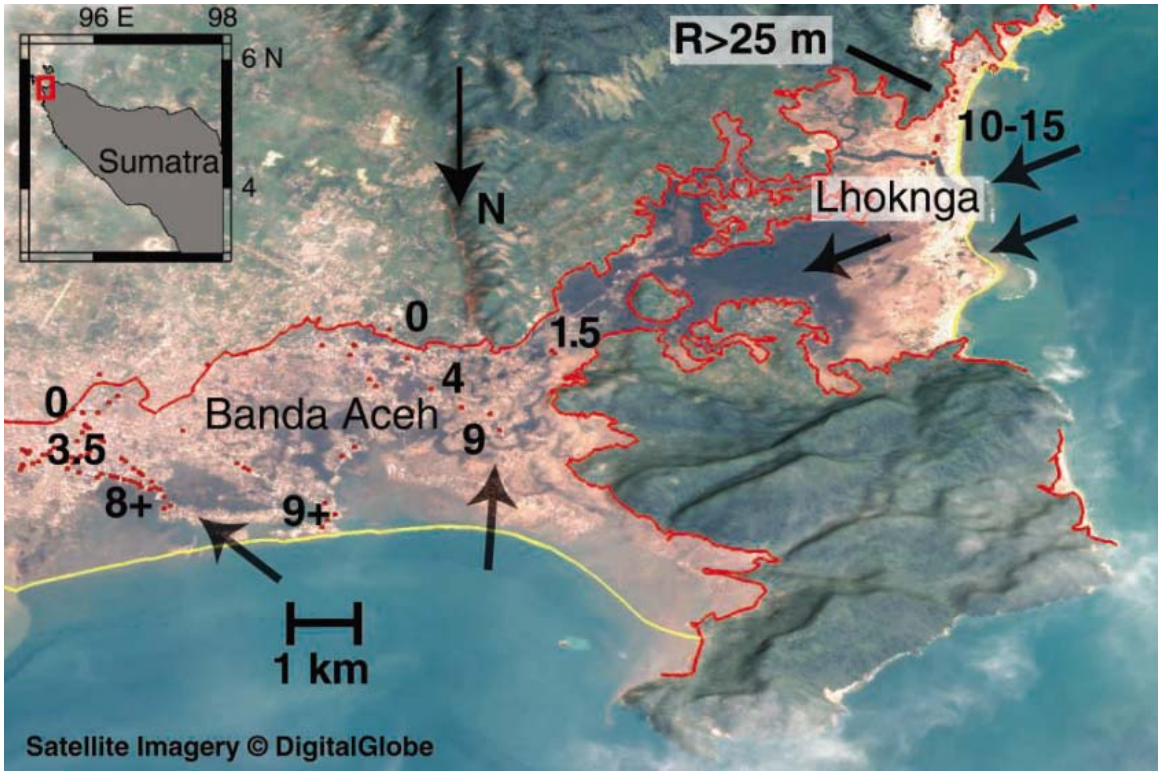

Fig. 1. Satellite image of Banda Aceh after the tsunami, draped over a 500-m digital elevation model. The yellow line indicates the shoreline before the tsunami; the red line identifies the extent of inundation. Red dots depict measurement locations of flow depth or direction. Numbers show representative measurements of flow depths (in m), and arrows show direction of flow. The tsunami flow depth was in excess of 9 m in Banda Aceh, and runup (R) ranged up to 31 m in Lhoknga.

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The Kinase Domain of Titin Controls Muscle Gene Expression and Protein Turnover

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The giant sarcomeric protein titin contains a protein kinase domain (TK) ideally positioned to sense mechanical load. We identified a signaling complex where TK interacts with the zinc-finger protein nbr1 through a mechanically inducible conformation. Nbr1 targets the ubiquitin-associated p62/SQSTM1 to sarcomeres, and p62 in turn interacts with MuRF2, a muscle-specific RING-B-box E3 ligase and ligand of the transactivation domain of the serum response transcription factor (SRF). Nuclear translocation of MuRF2 was induced by mechanical inactivity and caused reduction of nuclear SRF and repression of transcription. A human mutation in the titin protein kinase domain causes hereditary muscle disease by disrupting this pathway.

During muscle differentiation, a specific program of gene expression leads to the translation of myofibrillar proteins and their assembly into contractile units, the sarcomeres, which are constantly remodeled to adapt to changes in mechanical load. The giant protein titin (also known as connectin) acts as a molecular blueprint for sarcomere assembly by providing specific attachment sites for numerous sarcomeric proteins, as well as acting as a molecular spring (*1*, *2*). Titin also contains a catalytic serine-threonine kinase domain (TK), which is inhibited by a specific dual mechanism (*3*). However, the upstream elements controlling TK activation, its range of cellular substrates, and particularly the role of TK in mature muscle are largely unknown. Spanning half sarcomeres from Z disk to M band, titin is in a unique position to sense mechanical strain along the sarcomere (*1*). The elastic properties of the titin molecule and the mechanical deformation of the M band during stretch and contraction (*4*) suggest that the signaling properties of TK might be modulated by mechanically induced conformational changes. Molecular dynamics simulations suggest that mechanical strain can induce a catalytically active conformation of TK (*5*).

The catalytic kinase domain of titin interacts with nbr1. We searched for further elements of a putative signaling pathway that might recognize mechanically induced conformational intermediates of titin's catalytic domain. In a systematic two-hybrid screening approach with various structure-based open states of the catalytic site [kin1, kin2, and kin3 (*6*)], we identified the zinc-finger protein nbr1 (*7*) as a TK ligand, which interacted via its N-terminal PB1 domain with the semiopened construct kin3 (Fig. 1, A and B). This interaction was also seen in precipitation experiments with nbr1 and TK-kin3 (fig. S1A). Kin1, where the complete regulatory domain closes the active site, and kin2, where the α helix R1 (*3*) is deleted, did not interact. Thus, αR1 was necessary but not sufficient for nbr1 binding, which also required a semiopened catalyt-

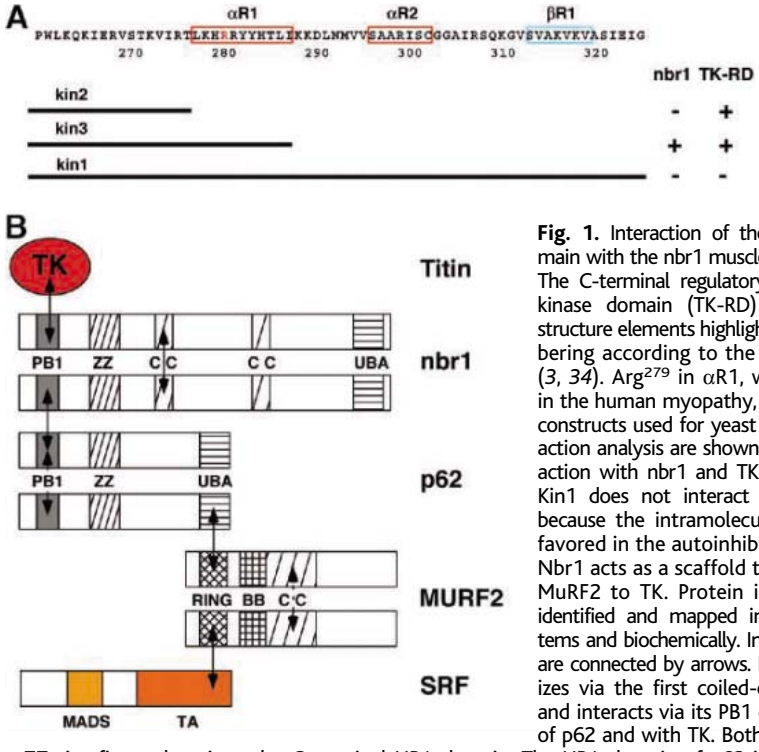

Fig. 1. Interaction of the titin kinase domain with the nbr1 muscle signalosome. (**A**) The C-terminal regulatory tail of the titin kinase domain (TK-RD) with secondary structure elements highlighted; residue numbering according to the crystal structure (*3*, *34*). Arg^{279} in αR1, which is mutated in the human myopathy, is highlighted. TK constructs used for yeast two-hybrid interaction analysis are shown below, and interaction with nbr1 and TK-RD on the right. Kin1 does not interact with the TK-RD, because the intramolecular interaction is favored in the autoinhibited form (*3*). (**B**) Nbr1 acts as a scaffold to target p62 and MuRF2 to TK. Protein interactions were identified and mapped in two-hybrid systems and biochemically. Interacting domains are connected by arrows. Nbr1 homodimerizes via the first coiled-coil domain (CC) and interacts via its PB1 domain with that of p62 and with TK. Both proteins contain a ZZ zinc-finger domain and a C-terminal UBA domain. The UBA domain of p62 interacts with the RING- and the B-box (BB) domain region of MuRF2, which can multimerize via a coiled-coil domain; in the cardiac isoform MuRF2p27, this domain is spliced out (*16*). MuRF2 in turn interacts with the last 54 residues of the SRF transactivation domain (TA).

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ic cleft. Such a conformational intermediate of TK activation is predicted to be mechanically inducible (*5*), suggesting that the nbr1 interaction with the regulatory domain of TK may channel into mechanically modulated signaling in muscle. PB1 domains provide protein-interaction modules in diverse signaling proteins, allowing the formation of large, heteromultimeric complexes (*8*). Because related zinc-finger proteins have been found to act as scaffolds for signalosome assembly (*9*), we searched for further nbr1-interacting proteins in muscle by yeast two-hybrid screens. We identified the nbr1-related zinc-finger protein p62 [also known as SQSTM1, ZIP, or ORCA (*10*, *11*)] as a further ligand of nbr1 in cardiac and skeletal muscle libraries (Fig. 1B). Both nbr1 and p62 were in vitro substrates of TK with substrate sites in the N termini (Ser^{115} or Ser^{116} for nbr1), although p62 was a significantly poorer substrate (K_M value is lower by ~10) than telethonin (*3*) or nbr1 (fig. S3).

The nbr1 ligand p62 interacts with the RING-B-box protein MuRF2. P62 is a multivalent scaffolding platform, which interacts with several kinase signaling pathways apart from that of TK (*11*). The ubiquitin-associated (UBA) domain of p62 is an important component of pathways controlling focal turnover in bone (*12*) as well as interacting with several signaling proteins in neurons (*13*).

We asked which interactions of p62 could channel TK signaling into muscle-specific responses. We identified the RING/B-Box protein MuRF2 as a ligand of the p62 UBA domain (Fig. 1B and fig. S2). Nbr1, p62, and MuRF2 proteins interacted in successive pairs in vitro and could be precipitated in a large complex from muscle tissue extracts, demonstrating their association in vivo (fig. S1B). MuRF2, a muscle-specific zinc-finger protein, is involved in primary myofibrillogenesis (*14*–*16*) and can shuttle between the cytosol and the nucleus under atrophic conditions (*16*). The closely related MuRF1 has been implicated in ubiquitin-controlled protein turnover in atrophic muscle (*17*) but also interacts with the ubiquitin-like modifier SUMO-3 (*18*) and the SUMO E2 ligase Ubc9 (*19*).

Because the interactions identified here suggest a link between TK and diverse signaling pathways, we assessed protein localization in muscle cells. Nbr1 and p62 were both detected at the sarcomeric M band (Fig. 2, A and B), together with TK (*20*) and MuRF2 (*16*). Thus, nbr1 appears to act as a muscle cytoskeleton-associated kinase scaffolding protein to assemble large sarcomeric signalosomes via its interactions with multiple proteins, linking TK to p62 and MuRF2. When nbr1 and TK kin3 were cotransfected into neonatal rat cardiomyocytes (NRCs), both proteins colocalized in sarcomeres (Fig. 2C). Overexpression of nbr1 perturbed normal localization and sequestered p62 (and to a lesser extent MuRF2) from M lines into cytosolic aggregates (fig. S2C). Thus, in muscle, nbr1 associates with TK and is essential for the correct targeting of the complex of p62 and MuRF2 to the M band.

TK-associated protein localization is mechanically modulated and regulates muscle gene expression. The interaction of nbr1 with a mechanically inducible conformation of TK, mimicked in TK kin3, led us to test the possible mechanical modulation of the localization of these proteins in muscle cells. NRCs are terminally differentiated and mechanically active but respond to stimuli modulating muscle-specific gene expression. Mechanical arrest of NRCs by various agents (*6*) resulted in marked nuclear accumulation of MuRF2 (Fig. 3, A and B) and the dissociation of p62 from the sarcomere and its relocalization to intercalated disks (fig. S4), suggesting that mechanical signals contribute crucially to regulating MuRF2 nuclear localization. To detect possible nuclear ligands of MuRF2, we performed two-hybrid interaction screening for MuRF2 ligands and detected an interaction of the RING/B-box domains with the transactivation domain of the serum response factor SRF (Fig. 1B). Activation of SRF-driven transcription of immediate-early genes like c-fos plays a central role in the response of muscle to hypertrophic stimuli including mechanical stress (*21*, *22*). SRF cooperates with other transcription factors in myogenic transcription [reviewed in (*23*)] and is crucial for heart development (*24*, *25*) and postnatal hypertrophic growth (*23*). The interaction with SRF suggests that MuRF2 might modulate muscle gene transcription by influencing SRF. In NRCs, we found that nuclear MuRF2 was associated with a strong reduction of the nuclear concentrations of endogenous SRF (Fig. 3, A and B) and its cytoplasmic accumulation. Similarly, overexpression of the small cardiac isoform MuRF2p27 alone in untreated NRCs resulted in a similar cyto-

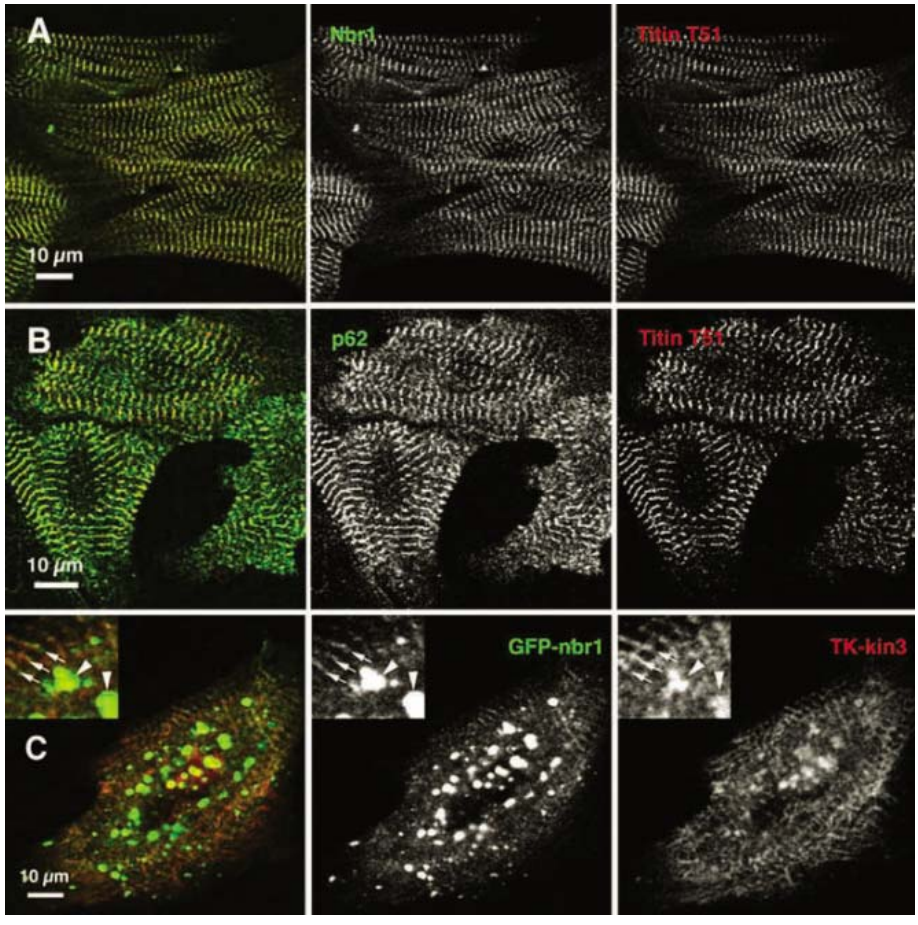

Fig. 2. Sarcomeric association of nbr1 and p62. (**A**) Confocal microscopy of neonatal rat cardiomyocytes demonstrates that nbr1 is a sarcomere-associated protein that colocalizes with the titin M-band epitope T51 (*20*), resulting in yellow color in the overlay (left images). Occasional Z-disk association can be observed additionally in skeletal muscle. (**B**) P62 is localized at the sarcomeric M band, similar to nbr1; it can additionally be found in a more diffuse pattern as well as at intercalated disks. (**C**) Cotransfection of TK-kin3 and GFP-nbr1 in NRCs shows both exogenous proteins localized together in sarcomeres (arrows) as well as in cytoplasmic aggregates (arrowheads). (Inset) Images magnified 2.6 fold.

plasmic localization of SRF (fig. S5A). When we assayed SRF transcriptional activity by reporter gene assays with the SRF-dependent c-fos promoter, we found that overexpressed MuRF2p27 led to strong suppression of c-fos activity (fig. S5B). Mutation of two zinc-coordinating cysteines (Cys^{29} and Cys^{78}) to alanine in the RING domain completely abolished nuclear localization of MuRF2. Nuclear MuRF2 therefore affects the nuclear pool of SRF in a RING domain–dependent way and represses transcriptional activity in muscle cells. Similar mechanically induced MuRF2 relocalization could also be observed in vivo, when the mechanical activity of skeletal muscle was arrested by denervation. MuRF2 appeared in the nuclei of sciatic denervated muscle fibers as early as 6 hours after mechanical arrest (up to 53% from under 4% in control fibers), suggesting this relocalization is an early event in denervation-induced atrophy (fig. S6).

Transfection of TK in NRCs leads to dissociation of MuRF2 from the sarcomere, nuclear exclusion, and strongly reduced MuRF2 protein amounts (Fig. 3C). Thus, TK activity can modulate MuRF2 protein concentrations as well as intracellular localization and causes opposite effects than mechanical arrest, leading us to test whether the overexpression of TK in myocytes would result in changes in muscle gene expression. We first tested this in the myogenic titin knock-out cell line BHK-Bi (*26*), which contains a homozygous deletion in the titin gene including the kinase domain, thus abolishing endogenous TK activity. The MYOM promoter drives expression of the M-band protein myomesin, a constitutive sarcomeric protein that is linked to myosin and titin with a constant stoichiometry to the thick filament proteins (*4*), serving as a reporter for sarcomere synthesis. The MYOM promoter contains various binding sites for both myogenic transcription factors like MyoD as well as for SRF, GATA, and NFκB (*27*) and there-

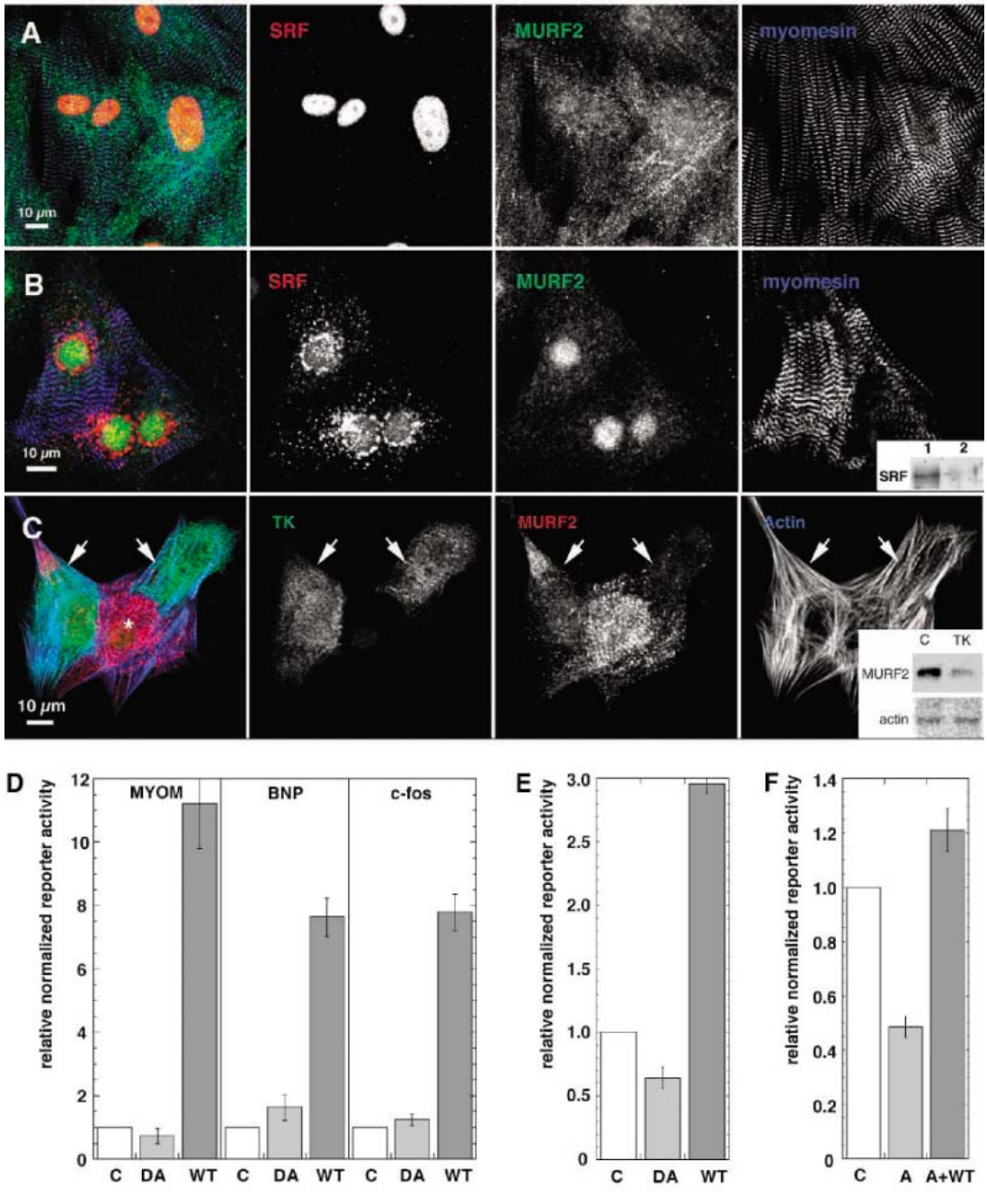

Fig. 3. The intracellular localization of MuRF2 is mechanically modulated. (**A**) In control NRCs, endogenous MuRF2 is associated with sarcomeres and SRF is nuclear. (**B**) Arrest of beating leads to nuclear translocation of MuRF2 and cytoplasmic accumulation of SRF; note reduced cell size. (Inset) Reduction of SRF in NRC nuclear extracts. Lane 1, control cells; lane 2, KCl-arrested cells. (**C**) In NRCs transfected with TK (arrows), MuRF2 concentrations are strongly reduced, and MuRF2 is excluded from the nucleus (asterisk, nontransfected cell). (Inset) Western blot (top row) confirms the reduced MuRF2 concentrations (bottom row, Ponceau Red–stained actin control band). C, mock-transfected control; TK, TK-transfected cells. (**D**) Transfection of TK in BHK-Bi cells stimulates transcription of myomesin (MYOM), BNP, or c-fos reporter genes. Mutation of the catalytic Asp^{127} to Ala^{127} (DA) abolishes activating effect. C, mock-transfected control; WT, wild-type TK. (**E**) MYOM reporter activity was assayed in NRCs cultivated in the presence of 20 μM phenylephrine (control, C). Transfected TK (WT) results in further stimulation of myomesin promoter activity, which is not observed for catalytically inactive TK (DA). (**F**) Influence of mechanical activity on muscle promoter activity. NRCs were cultured in presence of phenylephrine (mock-transfected controls, C). Arrest of contractile activity leads to reduction in myomesin promoter activity (arrested, A), which can be rescued by transfection of wild-type titin protein kinase (A+WT). Error bars indicate SEM.

fore could sense the combined input of the putative transcriptional regulation by TK-nbr1-p62-MuRF2 in muscle. We also tested the activity of the c-fos and the brain natriuretic peptide (BNP) promoter, markers of strain-regulated hypertrophy (*28*). In the titin knockout cell line BHK-Bi, exogenous TK activity led to strong increases of MYOM promoter as well as increases of c-fos promoter and BNP promoter activities (Fig. 3D). Similarly, in NRCs, which contain endogenous titin protein kinase activity, activation of promoters including MYOM was observed (Fig. 3E) even in presence of phenylephrine, an α-adrenergic agonist inducing cardiac hypertrophy. This suggests that TK and phenylephrine-activated α-adrenergic receptors signal via distinct pathways. Mechanical arrest of NRCs resulted in strong reduction of transcriptional activity including the MYOM promoter, even under persistent α-adrenergic stimulation (Fig. 3F). Overexpression of TK could rescue the mechanical arrest–dependent depression of muscle gene expression (Fig. 3F). Thus, the TK-nbr1-p62-MuRF2 pathway may be involved in mechanical signaling in muscle, in which strain modulated TK conformation and activity is channeled into the nbr1-p62-MuRF2 complex. Mechanical arrest would result in the relocalization of MuRF2 to the nucleus and of p62 to intercalated disks, whereas TK activity would counteract these changes and concomitantly induce the release of the inhibitory action of nuclear MuRF2 on SRF-mediated muscle gene expression.

A human mutation in the titin kinase domain disrupts nbr1 binding and leads to hereditary muscle disease. Support for the concept of a TK pathway feeding into muscle turnover via the nbr1-p62-MuRF complex comes from the analysis of hereditary myopathy with early respiratory failure (HMERF), an autosomal dominant muscle disease with proximal weakness of the upper and lower extremities and early involvement of neck flexors and respiratory muscles, causing respiratory failure as a frequent cause of death (*29*). At the ultrastructural level, abnormal Z disks and actin aggregates, with dissolving Z disk, I band, and M band structures, and radical focal myofibrillar breakdown are observed (*29*).

A genome-wide screen on two large unrelated Swedish families mapped the disease locus to chromosome 2q24-31 (*30*). Titin was the strongest positional and functional candidate gene. Sequencing revealed a heterozygous CGG→TGG change (Fig. 4A), leading to the exchange of a completely conserved arginine to tryptophan at position 279 (R279W) in αR1 of the TK regulatory tail (Fig. 4B). This showed complete cosegregation with the disease, and with a likewise segregating core haplotype, in the two families (Fig. 4C). This change was not reported in single nucleotide polymorphism databases and was not found in 200 (400 chromosomes) normal Swedish controls. Another Swedish patient with identical phenotype but without known genealogical relation to anyone in the two original families was found to have the same mutation on the same haplotype, indicating a common ancestry.

When recombinant R279W-mutant TK was assayed for calmodulin-stimulated catalytic activity, no significant change of activity in comparison to wild-type TK was observed (fig. S7). Because the R279W mutation in αR1 results in a drastic change of a surface-exposed basic to a nonpolar, bulky amino acid in the nbr1 binding site, we tested the interaction of TK with nbr1, which was dramatically reduced in the mutant TK (Fig. 5, A and B). In patient biopsies, nbr1 was localized abnormally diffusely in diseased muscle instead of being M band– and Z disk–associated, although in HMERF 50% of TK was expected to be wild type (Fig. 5, C and D). P62 accumulated in many diseased muscle fibers of the HMERF patients (Fig. 5, E and F). MuRF2 showed unusual nuclear localization in centralized nuclei of patient muscle fibers (Fig. 5G) not observed in peripheral nuclei of normal muscle or in three other myopathies with centralized nuclei (Duchenne muscular dystrophy, tibial muscular dystrophy, and myotubular myopathy). Furthermore, MuRF1, a close homolog of MuRF2, did not show nuclear localization in HMERF patients or in the other myopathies tested with centralized nuclei.

A knockout mouse model with a large deletion of M-band titin, encompassing TK and the binding sites for MuRF1/2/3, DRAL, and myomesin, is lethal when activated at embryonic stages and developed severe progressive muscle weakness at later stages of development (*31*). Similarly, a cell line with a targeted heterozygous deletion of the kinase exon leads to perturbed myofibril assembly (*32*). Both mutations affect numerous known protein binding sites in addition to TK itself, and their effects can therefore not be attrib-

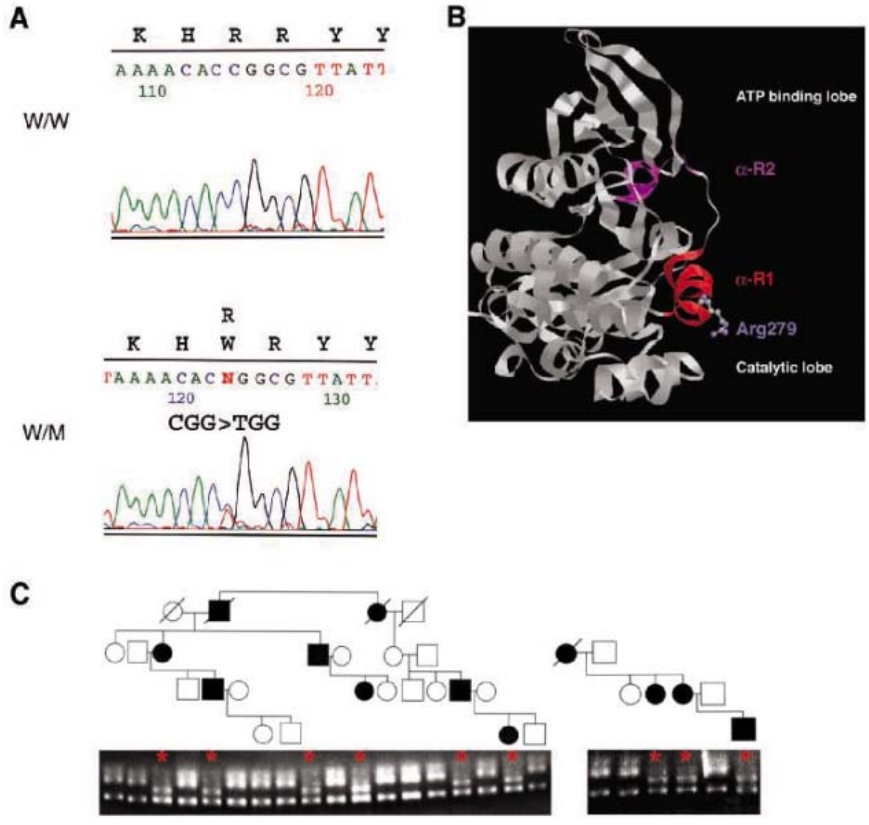

Fig. 4. A mutation in the human titin kinase domain causes a severe autosomal-dominant hereditary myopathy. (**A**) The single base pair exchange in αR1, leading to the exchange of R279 to W (*34*) as shown in the partial nucleotide sequences of heterozygous patient (W/M) and wild-type healthy control (W/W). The peptide sequence of αR1 is identical between the human, mouse, rat, and rabbit titin, arguing for crucially conserved interactions. Numbering of DNA sequence is arbitrary in these samples. (**B**) Ribbon diagram of the titin kinase domain with αR1 in red illustrates that the mutated residue R279 (side chain highlighted in blue) is a surface-exposed residue. (**C**) Pedigree of two Swedish families with HMERF; affected members are shown by solid symbols and unaffected by open symbols. (Bottom) Segregation of the titin protein kinase mutation is shown by Msp I restriction fragment length polymorphism analysis, and R279W heterozygous members are marked by red asterisks.

uted to abolished kinase signaling alone. In HMERF, primary myofibril assembly seems not to be affected. The severe pathology starts in adulthood, indicating an activity-related turnover or maintenance defect. The importance of tight regulation of kinase function for accurate turnover control is suggested by the fact that in HMERF a dominant mutation is pathogenic, despite an otherwise catalytically active enzyme and intact M-band titin. All reported cardiomyopathy patients with titin mutations lack skeletal muscle weakness, and TMD/LGMD2J and HMERF patients with titin mutations suffer from skeletal muscle weakness and atrophy without clinically dominant cardiac involvement. This may be caused by the expression of tissue-specific isoforms of MuRF or other components of the M band (*15*, *16*, *33*) in cardiac and skeletal muscle (fig. S8). The HMERF mutation we report here supports the suggested in vivo functions of the titin protein kinase as a physiological link between sarcomere activity and transcriptional regulation. Thus, the TK-associated protein complex may act as a central switchboard where input from various pathways leading to hypertrophic or stress response and protein turnover links the sarcomere to mechanical modulation of muscle gene transcription, a feedback mechanism that may prove to be important for therapeutic intervention in skeletal myopathies and heart failure.

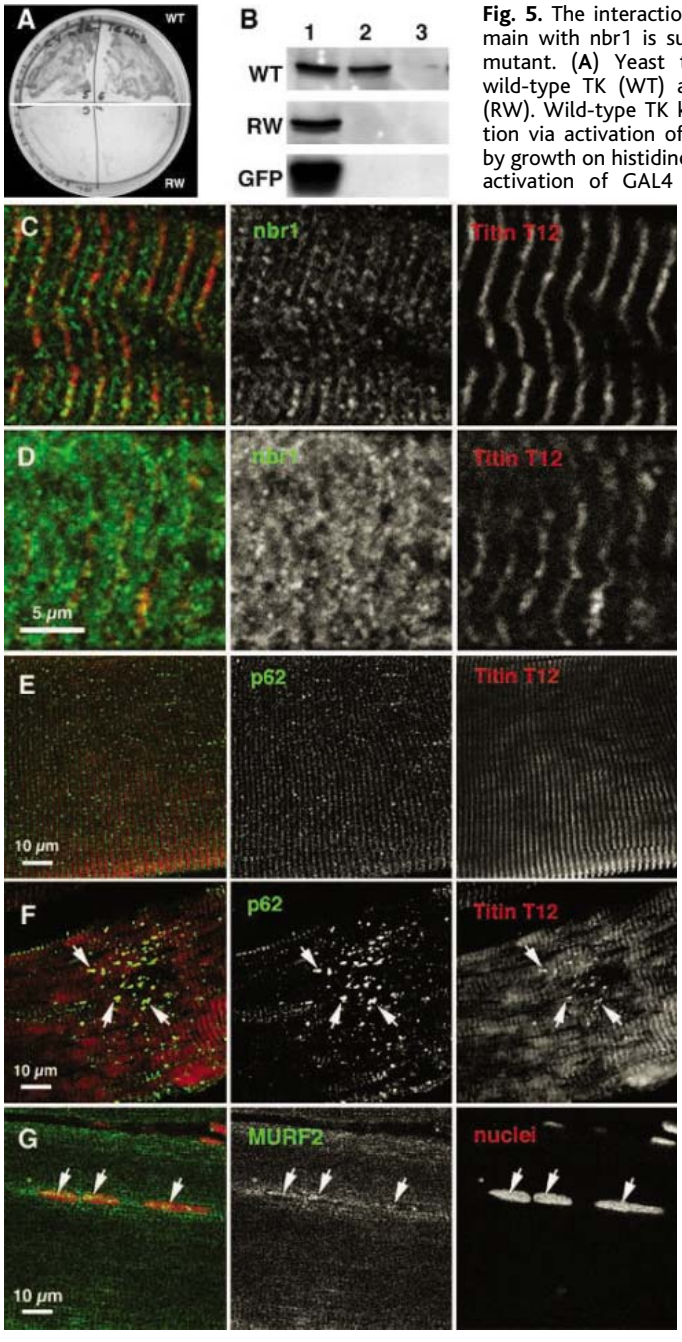

Fig. 5. The interaction of the titin kinase domain with nbr1 is suppressed in the R279W mutant. (**A**) Yeast two-hybrid assays with wild-type TK (WT) and the R279W mutant (RW). Wild-type TK kin3 shows nbr1 interaction via activation of the HIS3 reporter gene by growth on histidine-free medium, as well as activation of GAL4 β-galactosidase activity (not shown). Both markers for protein interaction are repressed in TK-R279W. (**B**) Precipitation binding assays demonstrate that TK-R279W fails to interact with glutathione *S*-transferase (GST)–nbr1, in contrast to wild-type titin protein kinase. Lane 1, 10% of input cell lysate; lane 2, eluted fraction from nbr1 beads; lane3, control beads with GST alone. Muscle biopsies from controls and HMERF patients were analyzed by immunofluorescence for nbr1, p62, and MuRF2. In normal skeletal muscle, nbr1 is mostly M band–associated, with occasional weaker Z disk association (**C**). Note the amorphous localization of nbr1 in the HMERF sample with preserved staining of the Z-disk titin epitope T12 (**D**). P62 is sarcomere-associated in normal muscle (**E**) but forms large cytoplasmic aggregates in HMERF (**F**), partly colocalizing with degraded Z-disk material stained by titin T12 (arrows). MuRF2 is localized aberrantly in centralized nuclei (arrowheads) (**G**) in HMERF muscle fibers.

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34. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1110463/DC1
Materials and Methods
Figs. S1 to S8
References

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Infrared Echoes near the Supernova Remnant Cassiopeia A

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Two images of Cassiopeia A obtained at 24 micrometers with the Spitzer Space Telescope over a 1-year time interval show moving structures outside the shell of the supernova remnant to a distance of more than 20 arc minutes. Individual features exhibit apparent motions of 10 to 20 arc seconds per year, independently confirmed by near-infrared observations. The observed tangential velocities are at roughly the speed of light. It is likely that the moving structures are infrared echoes, in which interstellar dust is heated by the explosion and by flares from the compact object near the center of the remnant.

Cassiopeia A (Cas A) is the youngest supernova remnant (SNR) known in our Galaxy and is thought to arise from the core collapse of a massive (~20 $M_{\odot}$) Wolf-Rayet star (*1*) about 325 years ago (*2*). Its youth and proximity to Earth ($3.4^{+0.3}_{-0.1}$ kpc, where the sub- and superscripts indicate lower and upper limits, respectively) (*3*) make Cas A a unique "laboratory" for supernova astrophysics. We imaged Cas A at 24, 70, and 160 μm on 30 November 2003 (*4*, *5*) with the use of the Multiband Imaging Photometer for Spitzer (MIPS) (*6*) in scan map mode. The observations yielded a rectangular image; a section about 40 arc min long by 10 arc min wide is displayed (Fig. 1A). At the distance of Cas A, 1.0 arc min corresponds to 1.0 pc or about 3 light years. The 24-μm image revealed fine filaments and knots that stand out clearly against the more diffuse galactic cirrus emission around the remnant. Two bright lobes of a bipolar structure are axisymmetrically located 14 arc min northeast and 11 arc min southwest from the center of the remnant (Fig. 1A).

We obtained near-infrared (λ = 2.2 μm) images of the northern lobe by using the Multiple Mirror Telescope (MMT) on Mount Hopkins (Arizona) on 30 May 2004 and the Calar Alto (Spain) 3.5-m telescope on 11 October 2004 and 8 January 2005. All three images show faint filamentary nebulosities within the region of the brightest 24-μm emission. These nebulosities undergo remarkable changes in morphology over the time interval of only a few months. One such filament is shown (Fig. 2), the emission region of which is moving at an angular tangential velocity of 18 ± 2 arc sec year^{-1}, close to the speed of light at the distance of the SNR.

We repeated our initial Spitzer scan map at 24 μm a year later, on 2 December 2004. The difference image (Fig. 1B) shows substantial changes out to the edge of our images about 25 arc min (80 light years) from the SNR. Two images within the northern lobe region (Fig. 3) are typical of the huge variations. The morphological variations make it difficult to follow distinct filaments over the 1-year time interval; however, there is a persistent ~7-arc-min-long filament (Fig. 1B) connecting the southern lobe of the bipolar structure with the remnant whose cusp exhibits a tangential velocity of $v \sim 0.7 \pm 0.1\ c$. There is a marked tendency for the motions in the bipolar structure to be outward from the center of the SNR. Our K-band images also show a number of features outside the bipolar structure with apparent motions in other directions.

High-velocity ejecta have previously been identified in optical emission lines (*7*) out to 4.5 arc min distance and with expansion speeds up to nearly 15,000 km s^{-1}. If the infrared (IR) features were produced by ejecta from the supernova explosion 325 years ago, their location would require average space velocities of at least a quarter of the speed of light, or 75,000 km s^{-1}, in addition to the observed apparent velocities close to the speed of light. Such velocities have never been observed from any individual ejecta in supernova remnants, nor have they been spectroscopically inferred from supernova outbursts. Apparent motions close to the speed of light have, however, been observed in scattered light and IR echoes originating from supernova explosions (*8*, *9*) and other brilliant light sources (*10*, *11*).

We believe that IR echoes, i.e., the heating of interstellar dust by the outward moving photon shell of a bright flash and the echoed reradiation of the resulting IR emission (*12*), provide the most convincing interpretation for the moving IR features near Cas A. This hypothesis is supported by images of the northern lobe at 3.6, 4.5, 5.8, and 8 μm obtained with Spitzer on 17 December 2004 and at 2.2 μm from Calar Alto on 8 January 2005, which accompany the 2 December ones at 24 μm. The morphologies in all these bands are similar, allowing us to identify individual features reliably. The average spectral energy distribution (SED) (Fig. 4) is consistent with emission dominated by a smooth, but very red, continuum (but with some spectral structure across the 3.6 to 8 μm region) and suggestive of thermal emission from warm dust. The reddening toward Cas A ($A_K \sim 1$) is insufficient to change the overall shape of the SED substantially. A direct scattered light echo is unlikely because it would be blue in color (*8*), in contrast to the observed red continuum.

A thermal dust continuum is also consistent with the lack of line emission (3 σ upper limit of 60 μJy; 1 Jy = 10^{-26} W m^{-2} Hz^{-1}) in K-band spectroscopy (spectral resolution $\lambda/\Delta\lambda \sim 500$) of two bright emission peaks obtained on 30 July 2004. Line emission is also not detected in narrow band images of the northern lobe region in filters for H_2 (2.12 μm) and Br γ emission obtained on 8 and 11 October 2004. We can identify the lobe to the southwest in our 70-μm image at a level consistent with thermal emission. However, confusion with bright galactic cirrus emission and the lower spatial resolution at this wavelength complicate an unambiguous determination of the 70-μm flux density for the moving features. A bright filament wisp was faintly detected in a deep R band image obtained on 27 December 2003; because of the uncertainty in the extinction, we have not tried to include this point in the SED.

Emission by an IR echo is also consistent with the nondetection of the filaments in the radio continuum and at x-ray energies. No trace of the filaments was detected at cm wavelengths toward three fields shown in Fig. 1 with the use of the Very Large Array (VLA) in BnA configuration at C band (4.9 GHz) and X band (8.4 GHz) on 6 and 7 February 2005. For these fields, from north to south, the root mean square noise levels at C band were 40, 66, and 80 μJy [beam size of 0.9 × 0.5 arc

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sec^2 full width at half maximum (FWHM)]; and at X band were 56, 60, and 81 μJy (beam size of 1.6 × 0.9 arc sec^2). This is far below (less than one-thousandth) a traditional synchrotron power law spectrum (spectral index = –0.7) normalized at 24 μm and argues against a relativistic jet being responsible for the apparent superluminal motions (*13*). The Chandra High Resolution Camera (HRC) imaged Cas A on 19 December 1999. The field of view covered the two inner circled regions in Fig. 1, about 12 arc min to the north and south of the SNR. No significant x-ray emission was detected within either of the circled regions to an unabsorbed flux limit of 1×10^{-13} erg cm^{-2} s^{-1} (0.2 to 6 keV band). Given the variability of these features, it would be desirable to confirm this result with more contemporaneous x-ray and IR measurements.

If the heating flash for the IR echoes was emitted by the supernova explosion, then the structures we see now must lie 50 to 60 pc behind the remnant to account for the delay of 325 years in light travel time. The angle of incidence of the flash relative to the plane of the sky would be about 75°, and as a result there would only be a mild tendency for an IR echo to prefer outward motion. Although some of the IR features with nonsystematic motions may arise from dust heated by the initial explosion, the strong observed outward tendency of the IR echoes in the bipolar structure suggests the presence of an object within the remnant that has recently had short outbursts. The sharp appearance of the infrared features, which are partly unresolved in our K-band images at 0.7–arc sec resolution, indicates that the time scale of such an outburst and the subsequent dust cooling can be no more than a few weeks [The expected cooling time scales for typical interstellar dust particles are substantially shorter than this limit (*14*)]. The geometry of the echoes is consistent with heating by short flares of beamed light emission roughly perpendicular to the line of sight from the central compact x-ray source (*15*), whose precise nature is unknown. Assuming that the two lobes northeast and southwest of the remnant correspond to IR echoes of one such beamed flare, we can determine its date and space direction from the position of the sharply defined outer lobe boundaries relative to the central object. Equating the light travel time to the northeast lobe (14.0 ± 0.5 arc min) and the southwest one (11.3 ± 0.5 arc min) yields a flare date of A.D. 1952.9 ± 2.5, at position angle 26 ± 4° on the sky and at an angle of 82 ± 3° relative to the line of sight. The flare may have escaped direct detection because it was beamed nearly perpendicular to the line of sight.

Strongly magnetized neutron stars (magnetars) localized in young supernova remnants (*16*) are known to emit short, nonperiodic, and luminous flares primarily detected at high photon energies (*17*), in which case they are described as soft gamma ray repeaters (SGRs). Giant flares from SGRs are also accompanied by outbursts of relativistic particles (*18*, *19*). The position angle of the 1952 flare coin-

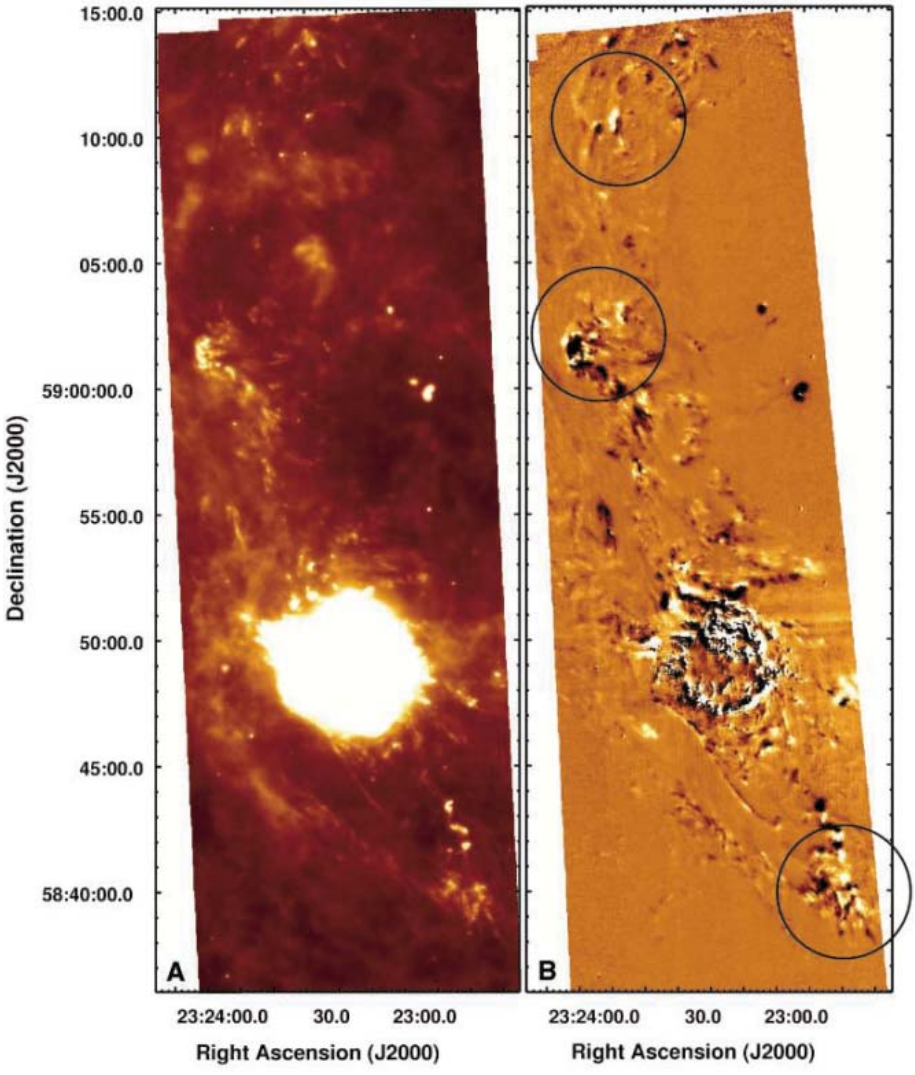

Fig. 1. (**A**) MIPS scan map image of Cas A taken on 30 November 2003. Colors represent 24 μm surface brightness (range from 18 to 35 MJy sr^{-1}). (**B**) Difference image of Cas A at 24 μm. Black features correspond to the MIPS scan map on 30 November 2003, and white features correspond to observations on 2 December 2004. The location and size of the VLA fields are indicated by circles. The image components due to the stationary IR cirrus cancel out very well in the difference image (e.g., to the northwest and southeast of the SNR).

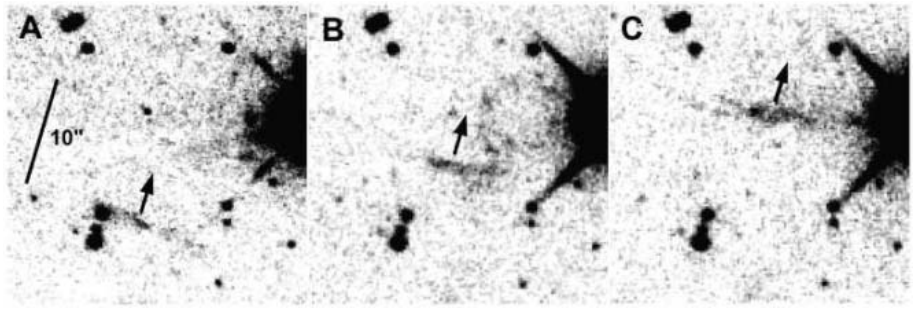

Fig. 2. K_s-band images of a moving filament in the northern lobe region obtained in May 2004 from MMT (**A**) and in October 2004 (**B**) and January 2005 (**C**) from Calar Alto. The field size is ~ 30 arc sec by 30 arc sec. The 10–arc sec scale in (C) corresponds roughly to the light travel distance during the ~7-month interval shown here. The seeing for all three images was about 0.7 arc sec.

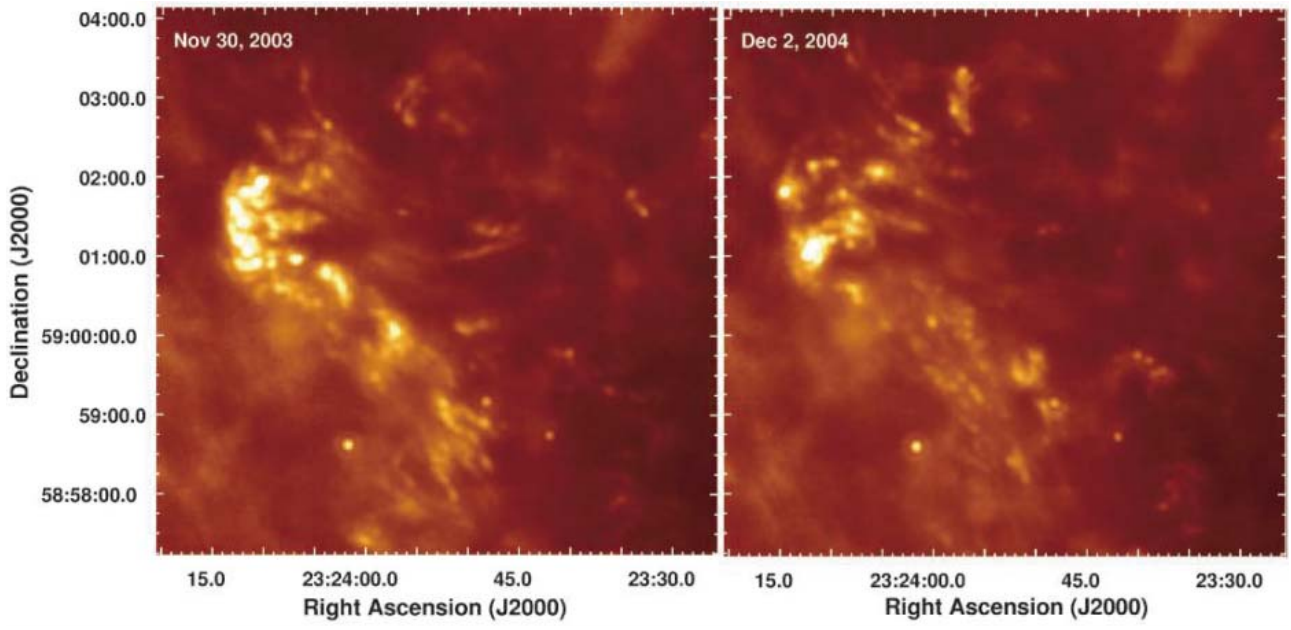

Fig. 3. 24-μm MIPS images of the northern lobe region. Large proper motions and morphological variations occurred in the filamentary nebulosity over the 1-year interval.

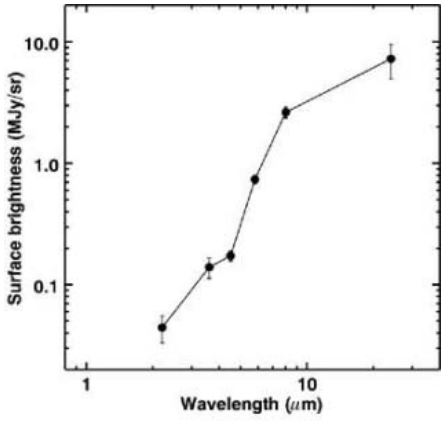

Fig. 4. Average spectral energy distribution of the infrared features in the northern lobe region. Images at 3.6, 4.5, 5.8, and 8 μm from Spitzer and at 2.2 μm from Calar Alto were convolved to match the MIPS 24-μm beam. The surface brightness was then measured point by point at 59 positions in the Nyquist-sampled images after removal of field stars and diffuse cirrus emission. The SEDs for each of the statistically independent positions were normalized to their average. The quoted 1 σ uncertainties include the standard deviation of the individual normalized data points in each band and their systematic calibration errors.

cides with the location (position angle ~ 26°) of the radio structure H (*20*), which is one of the outermost bright features of Cas A. Radio observations in 1974 revealed a compact radio knot within this structure (*21*), which was not visible in 1969. In case the enhanced synchrotron emission of this feature is powered by relativistic particles from the ~1952 flare, their average space velocity would have to be $v \sim 0.6$ c to have reached the position of the radio knot between 1969 and 1974. This is consistent with the inferred velocity of ablated baryons in a recent SGR flare (*19*).

The x-ray point source near the center of the remnant is believed to be a neutron star. Although no soft gamma ray bursts have ever been detected from it, its spectral properties are consistent with a momentarily quiescent SGR (*22*). Alternatively, it has been suggested that the central object is in the process of evolving into a SGR (*23*). Although the status of this object remains uncertain, we take the properties of SGRs as a guideline in a rough calculation of luminosity. SGR giant flares can have isotropic energy equivalents of 10^{44} to 5×10^{46} erg (*17*, *24*); however, there are indications that their emission might be anisotropic (*25*). The bolometric IR luminosity of the features in the northern lobe region is ~ 0.02 $L_\odot$ arc sec^{-2}, or about 0.6 $L_\odot$ within our 6–arc sec (FWHM) beam at 24 μm. We hypothesize that the IR features represent the effect of a light pulse passing through the interstellar gas, which instantaneously heats dust it encounters along an expanding sphere centered on the neutron star. Assuming the northern lobe to represent a tangent position to this sphere, the time for the heated region to transit the 6–arc sec beam is about 4 months. A flare of isotropic energy equivalent to 2×10^{46} erg absorbed entirely over this time period would yield a net luminosity of ~ 60 $L_\odot$ within the 24-μm beam, about 100 times more than observed. The luminosities of SGRs therefore appear consistent with the heating requirements for the IR features, even accounting for a possibly low filling factor of the absorbing material.

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Resonant Optical Antennas

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We have fabricated nanometer-scale gold dipole antennas designed to be resonant at optical frequencies. On resonance, strong field enhancement in the antenna feed gap leads to white-light supercontinuum generation. The antenna length at resonance is considerably shorter than one-half the wavelength of the incident light. This is in contradiction to classical antenna theory but in qualitative accordance with computer simulations that take into account the finite metallic conductivity at optical frequencies. Because optical antennas link propagating radiation and confined/enhanced optical fields, they should find applications in optical characterization, manipulation of nanostructures, and optical information processing.

Efficient interconversion of propagating light and localized, enhanced fields is instrumental for advances in optical characterization (*1*–*5*), manipulation (*6*–*8*), and (quantum) optical information processing (*9*–*13*) on the nanometer scale. This requirement recently triggered a search for favorable structures (*1*–*5*, *13*–*21*) and materials (*10*, *22*). Resonant optical antennas excel among other structures by combining (i) field-line concentration at a local shape singularity, that is, a gap (*2*, *3*); (ii) optimum impedance matching to freely propagating waves; and (iii) resonant collective oscillations (plasmons) of the free electron gas (*4*, *5*, *11*, *21*) in the antenna arms. While the field enhancement in the feed gap obviously increases with decreasing width (*20*), variation of the overall antenna length should result in a pronounced resonance in analogy to the radio wavelength regime.

We demonstrate that gold dipole antennas can be designed and fabricated to match optical wavelengths. Upon illumination with picosecond laser pulses, white-light supercontinuum (WLSC) (*23*–*25*) radiation is generated in the antenna feed gap in addition to two-photon photoluminescence (TPPL) (*20*, *26*, *27*) in the antenna arms. The emission from the antennas is more than 10^3 times as strong as that from solid gold stripes of the same dimensions but without feed gap. Variation of the overall length of the antenna reveals a resonance substantially below one-half of the effective excitation wavelength.

The nanometer-scale dimensions of resonant optical antennas raise a twofold experimental challenge, that is, manufacturing with sufficient precision and identification of specific antenna effects. The first challenge can be met by means of modern microfabrication techniques, demonstrated for bow-tie antennas at infrared and, more recently, at optical frequencies (*15*, *16*, *19*, *20*). We fabricated slim dipole antennas with lengths in the half-wavelength range by means of focused-ion beam (FIB) milling. In an initial step, sets of stripes with full length L = 190 to 400 nm and width 45 nm were cut from 40-nm-thick, micrometer-sized rectangular gold patches arranged well separated from each other on an indium tin oxide (ITO)–coated (10-nm thickness) glass cover slide. For complete gold removal, the FIB had to cut slightly into the substrate, leaving a shallow depression (~20 nm) around the stripes. In the final step, one-half of the stripes were converted into optically resonant antennas (ORA) by cutting a narrow groove through the centers of the stripes, leaving an ~20-nm-wide gap between the ORA arms (Fig. 1, A and B). "Resonant" here refers to the laser excitation rather than to WLSC emission, because the permittivity of the substrate and the finite thickness of the antennas reduce the effective wavelength at the interaction zone to values considerably off resonance.

Specific antenna effects were identified with picosecond laser pulses powerful enough to excite WLSC in addition to TPPL and by comparing explicitly the responses of ORAs and stripes. TPPL is a second-order process well documented for gold (*20*, *26*, *27*). WLSC is a fourth-order optical nonlinearity found in various dielectric materials such as glass (*23*, *25*) and water (*24*) but not in gold (*20*, *26*, *27*). WLSC hence provides information on the field enhancement outside the ORA arms. The mechanisms underlying WLSC are not well known but seem to require a minimum pulse length in the picosecond range. Both mechanisms contribute to the "white-light continuum" (WLC) recorded in our experiment, the respective contributions being distinguished by their spectral features and power dependences.

The sample was mounted in an inverted optical microscope modified for confocal operation in reflection (fig. S2A). Laser pulses [center wavelength λ_o = 830 nm, repetition rate 80 MHz, pulse length 8 ps at the sample (figs. S2B and S4), maximum average power 150 μW, which is more than a factor of 5 below damage threshold] were focused (1.3 NA, ∞) to a diffraction-limited spot on the sample. The polarization at the sample was linear and adjustable in direction. The pulses were spectrally cleaned with a line filter before entering the microscope and blocked with a notch filter in front of the detectors. WLC spectra and power dependences of individual structures were obtained at fixed sample positions. WLC emission maps were recorded by scanning the sample with a single-photon-counting avalanche diode (SPAD) detector in combination with an additional bandpass filter (450 to 750 nm) (Fig. 1, C and D). The coincidence of the emission spots with the positions of ORAs and stripes was confirmed with a precision of better than 100 nm by comparison with large-scale scanning electron microscope (SEM) images and optical images (fig. S1). Sizeable WLC emission was found only at the positions of ORAs, for ORAs of a certain length range, and for ORA orientation along the pump polarization (e.g., Figure 1C, antenna #12). The emitted light is polarized along the ORA main axis as well, independent of the excitation polarization, which indicates the importance of the ORA also for the process of WLC emission (fig. S3). For comparison, signals from stripes are barely detectable (Fig. 1C, antenna #16) and are frequently associated

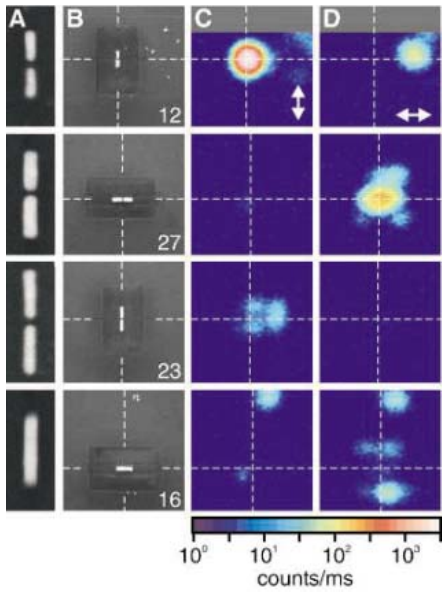

Fig. 1. Examples of ORAs and of a stripe. (**A** and **B**) SEM images, zoom and overview, respectively. (**C** and **D**) Confocal scan images of the WLC generated by vertically and horizontally polarized laser pulses, respectively (average power 110 μW, logarithmic color code). Dimensions: (A) 450 × 180 nm²; [(B), (C), and (D)] 2 × 2 μm². An overview of all ORAs and stripes investigated (including enumeration) is presented in fig. S1.

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with WLC generation at the rims of the depressions mentioned above.

The WLC spectra (Fig. 2) extend over a considerable range on both sides of the laser line, independent of antenna length. We concentrate here on the short-wavelength wing. At low power, the intensity falls off monotonously toward short wavelengths, typical for TPPL of gold (*26*, *27*). At high power, the spectrum is dominated by a broad peak around 560 nm that we assign to WLSC. Figure 3 shows the dependence of WLC power on laser power for ORAs of different lengths. The log/log curves first rise with slope 2 (dashed lines), which is typical for TPPL. For high-power excitation, the curves follow a fourth-order power law (dashed-dotted lines), supporting the above assignment to WLSC. The nonlinearity of the WLC process was used to determine the laser pulse length right at the position of an ORA by means of the autocorrelation technique (fig. S4). Both Schuck *et al*. (*20*) and Beversluis *et al*. (*27*) report second-order behavior only, although the same materials and a similar range of excitation power are applied. The main differences from the present experiment are the use of femto- instead of picosecond laser pulses and of structures of a different, possibly less favorable, shape.

The variation of WLC power with ORA/stripe length (L) is displayed in Fig. 4A (see also fig. S5) for low- and high-power excitation corresponding to a dominance of TPPL and WLSC, respectively. For the shortest and longest lengths, the antenna emission is about 10 times as strong (30 μW) and 100 times as strong (110 μW), respectively, as the emission of the corresponding stripes. The antenna emission goes through a maximum in between, whereas the stripe emission hardly varies over the whole range of lengths. The ratio of emission intensities reaches values as large as ~30 (antenna #17, L = 258 nm) and ~2000 (antenna #12, L = 250 nm), respectively. The emission data scatter considerably between individual antennas, although Fig. 4A includes only structures that showed a high degree of perfection in the SEM (fig. S1B). This suggests that the WLC emission might be influenced also by material imperfections not visible in the SEM.

We computed the near-field intensity (|electric field|^2) enhancement 10 nm above ORAs and stripes versus length L in steps of 20 nm, using Green's tensor technique (*28*). Figure 4, B to D, reveals drastic differences between antennas and stripes of the same length as well as between antennas of different lengths. This refers to both spatial distribution and amplitude of the enhancement. The strong field concentration in the feed gap of the resonant antenna (Fig. 4C) suggests that WLSC is generated mainly in the underlying ITO/glass substrate, and possibly also in water that might condense inside the gap. Further increase of the enhancement may be expected from reduction of the feed gap width (*20*, *29*).

As a figure of merit for the antenna response, $R(L)$, we use the near-field intensity, integrated, that is, averaged over the whole antenna area plus its immediate environment (600 × 200 nm^2). Although this is a somewhat arbitrary choice, the similarity of $R^2(L)$ and $R^4(L)$ with the experimental data is apparent, showing a flat response for the stripes but a pronounced peak for the ORAs for the same antenna length as in the experiment (Fig. 4A). No fit parameters were used in Fig. 4A except for a scaling factor.

We identify the L_0 ~ 255-nm maximum, seen in experiment and simulation, with the so-called half-wave dipole resonance that might as well be considered a plasmon mode with strong field concentration in the feed gap (Fig. 4B). The latter effect is specific for the optical regime where the permittivity of gold is small (*30*). This prevents the design of ORAs by simple downscaling of radio-wave antennas. WLSC originating from the feed gap volume provides unusual illumination properties that may allow for new forms of local spectroscopy (for instance, single-molecule Raman) and interactions with nanostructures and single-quantum systems.

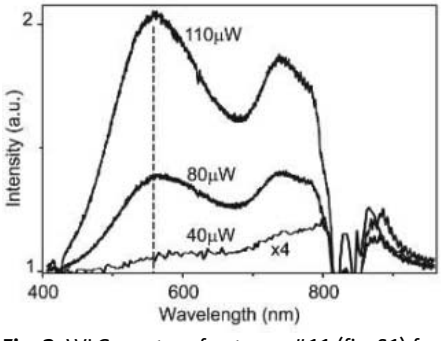

Fig. 2. WLC spectra of antenna #11 (fig. S1) for different excitation powers. Note the increase of the peak at 560 nm for increasing excitation power.

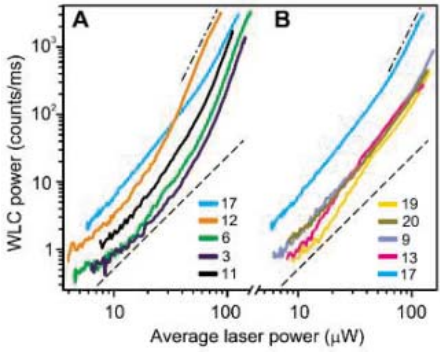

Fig. 3. WLC power dependence of ORAs with different lengths, grouped according to the dominant nonlinearity. (**A**) Fourth order, dashed-dotted line. (**B**) Second order, dashed line.

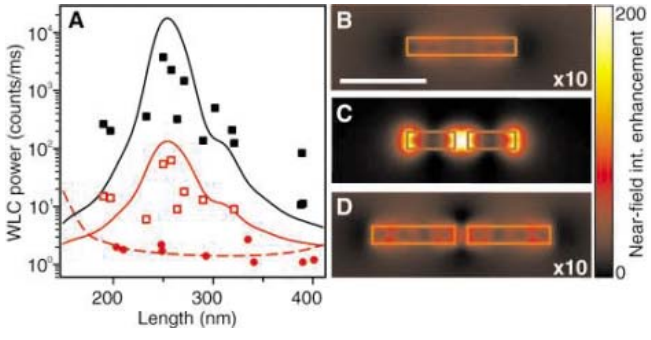

Fig. 4. (**A**) Variation of WLC power with antenna/stripe length. Filled and open squares, ORA at 110 and 30 μW, respectively; circles, stripe at 110 μW (fig. S5); solid red and black curves, $R^2(L)$ and $R^4(L)$ for ORAs, respectively; dashed line, $R^2(L)$ for stripes. (**B** to **D**) Near-field intensity (|electric field|^2) enhancement factor computed 10 nm above a stripe (250 × 40 nm^2), a resonant antenna (250 × 40 nm^2), and an off-resonant antenna (410 × 40 nm^2), feed gap 30 nm, gold on glass, λ = 830 nm, ε = –25.3 + i1.6 (*30*) and 2.25, respectively. Enhancement factor refers to |electric field|^2 of an evanescent field in the absence of the antenna. Scaling factor in (B) and (D), 10x. Scale bar, 200 nm.

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Supporting Online Material
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Figs. S1 to S5

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Giant Larvacean Houses: Rapid Carbon Transport to the Deep Sea Floor

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An unresolved issue in ocean science is the discrepancy between the food requirements of the animals living on the deep sea floor and their food supply, as measured by sediment traps. A 10-year time-series study of the water column off Monterey Bay, California, revealed that the discarded mucus feeding structures of giant larvaceans carry a substantial portion of the upper ocean's productivity to the deep seabed. These abundant, rapidly sinking, carbon-rich vectors are not detected by conventional sampling methods and thus have not been included in calculations of vertical nutrient flux or in oceanic carbon budgets.

Most deep benthic communities are supplied with food by a process described more than a century ago as a "rain of detritus" (*1*). The vertical flux of organic carbon in small particles, fecal pellets, and aggregates of marine snow is typically measured by sediment traps (*2*). Most of the particles that reach the deep sea floor are less than 5 mm in size, sink slowly, and have organic carbon levels that are reduced by microbial mineralization during their descent, which may last for months (*3*, *4*). Pulses of small particle flux are coupled to surface productivity (*5*–*7*). In studies of the relationship between organic carbon flux and the nutritional requirements of the deep benthic fauna, there is a discrepancy between the amount of food used by these animals and what can be accounted for by sediment traps on the supply side (*8*–*10*). This gap may be linked to declines in productivity that have accompanied the recent warming of the upper ocean (*9*, *11*–*13*). A number of secondary sources have been suggested that might make up the difference between supply and demand, including carrion falls, pulses of phytodetritus, and lateral transport from continental shelves (*9*, *14*–*16*). All of these probably contribute to the deep benthic food supply, but none have been shown to occur in sufficient quantity or with the consistency necessary to compensate for the disparity.

Here we discuss a class of particles consisting of the large, discarded feeding structures of giant mesopelagic larvaceans (appendicularians). These planktonic tunicates feed on suspended particles by secreting intricate filtration structures made of mucopolysaccharides (Fig. 1A), through which they pump water by beating their tails (*17*). An active filter structure is called a "house" because the animal lives inside it. Typically, each house has two nested filters: a coarse outer mesh and a fine-mesh inner structure. Giant larvaceans attain lengths up to 60 mm, and their houses are frequently greater than a meter in diameter (*17*, *18*).

The first giant larvacean identified, *Bathochordaeus charon*, was discovered in 1898, but their feeding structures were unknown until the 1960s, when they were observed during submersible dives (*18*). Subsequently, giant larvacean houses have been reported by observers using undersea vehicles in the eastern and western Pacific and in the Atlantic (*17*, *19*, *20*). These large houses are very fragile and do not survive capture by plankton nets. As a consequence, their potential contribution to vertical carbon flux was not recognized until they were observed in situ (*21*).

Larvacean houses are disposable, and when one becomes clogged with particles, the animal simply discards it and makes another. The structures collapse when water is no longer pumped through them (Fig. 1B). Once abandoned, they sink rapidly to the sea floor at a rate of ~800 m day^{-1} (*17*). At this rate, there is little time for mineralization by microbes. Discarded houses have not been accounted for by conventional methods for sampling sinking detritus (*22*), and thus their contribution to nutrient flux has not been factored into oceanic carbon budgets (*23*).

We used remotely operated vehicles (ROVs) to measure the abundance of both occupied and discarded giant larvacean houses (called "sinkers") and to collect them for chemical analyses. Abundance was measured by quantitative video transects at 100-m depth intervals, down to 1000 m, on about a monthly basis from 1994 through 2003. By calibrating a camera to record a measured area and then measuring the distance traveled during each transect, we were able to examine a known volume of water at each depth (*24*).

Samples for chemical analysis were collected with specialized samplers by skilled pilots, who carefully positioned the open containers around the delicate sinkers, then gently sealed them inside. Because the sinkers are so very easily fragmented and dispersed, only about 1 in 4 of our collection attempts was successful, and it is easy to see how sediment traps have missed them (*25*). As the sinkers descend, hydrodynamic forces shape them into increasingly compact forms (Fig. 1C); nevertheless, they remain easily disrupted by mechanical contact.

We surveyed the water column at three sites along the axis of the Monterey Canyon, off the California coast. These direct observations revealed a distinct class of large sinking aggregates, clearly derived from giant larvacean houses. The midwater fauna off Monterey Bay contains at least three giant larvacean species, each with a characteristic depth range and a large (>30 cm in diameter), distinctive house (*26*–*28*). The abundance of occupied houses and sinkers varied seasonally and interannually, but both were present year-round (Fig. 2). Estimates of the house-production rate of *Bathochordaeus* range from one per day (*16*) to one per month (*17*). On the basis of our counts of occupied houses, sinkers, and their sinking rate, we calculate that *Bathochordaeus* produces a new house every day (*24*) (Fig. 3). Sinkers are commonly observed during dives along the floor of the Canyon, with densities as

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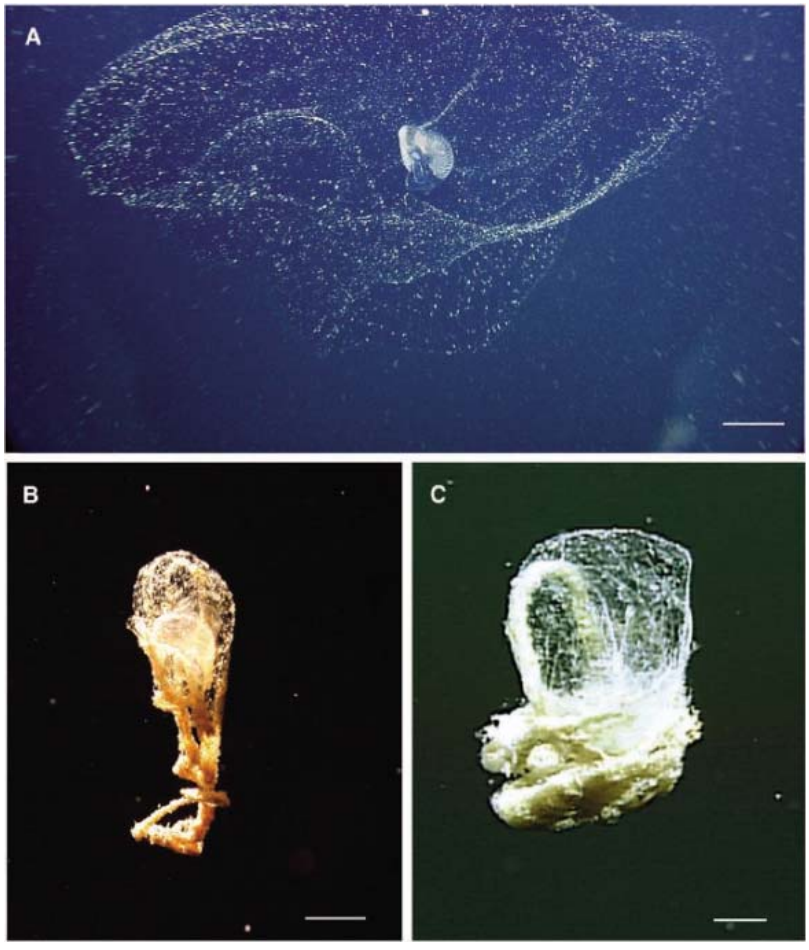

Fig. 1. In situ video frame grabs of steps in the progression from an actively filtering giant larvacean house to a descending sinker. (**A**) An active house occupied by *Bathochordaeus*; the coarse mesh outer filter surrounds a fine mesh inner filter, to which the tadpole-shaped animal is attached. (**B**) An abandoned and collapsed house, with most of the outer filter condensed into ropy strands and a small portion domed over the inner filter. (**C**) As the sinker rapidly descends, the mass becomes more compacted, and the inner filter is usually the last part to collapse. Scale bars: (A) and (B), 10 cm; (C), 1 cm.

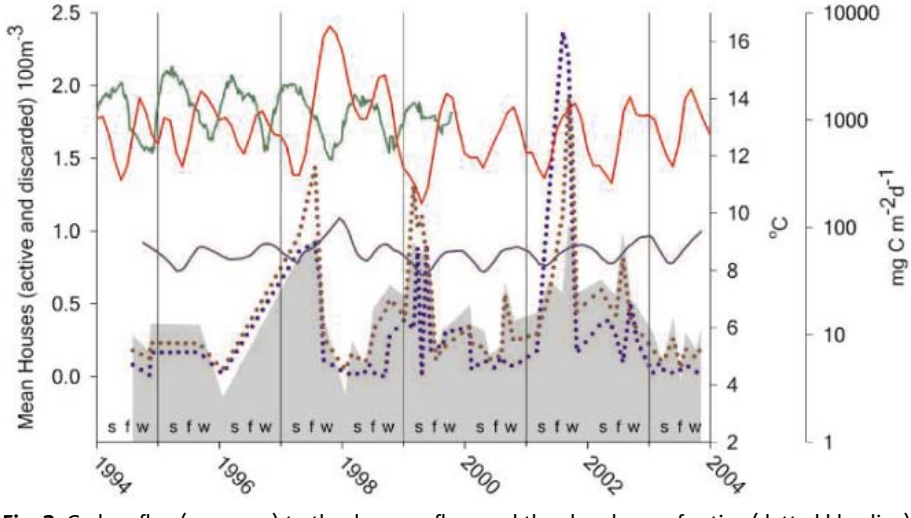

Fig. 2. Carbon flux (gray area) to the deep sea floor and the abundance of active (dotted blue line) and discarded (dotted red line) giant larvacean houses. Data collected in a 10-year ROV-based time series in Monterey Bay, California, show a consistent supply of carbon over summer (s), fall (f), and winter (w) seasons. Integrated primary productivity values (green line) and temperatures (at the surface, solid red line; at 200-m depth, solid blue line) were taken at a permanent mooring adjacent to the transect site (*32*). A negative exponential (second-degree polynomial function) was used to smooth the temperature and integrated carbon data.

high as 1 sinker per m^2 (*17*). Over the 10-year span of this study, the average flux of sinkers to the sea floor was 3.9 m^{-2} day^{-1}. We measured the particulate organic carbon (POC) and dissolved organic carbon content of 105 sinker samples, collected over a 2-year period at depths from 200 m to 2979 m (*24*). The average of total organic carbon was 5.4 mg, and the average C:N ratio was 6.09.

When we calculate nutrient flux by multiplying the average organic carbon content of a sinker by the number reaching the bottom each year, we get a rate of 7.6 g of C m^{-2} $year^{-1}$ (Fig. 2). Data from sediment traps deployed in the same region as our dive sites have shown annual carbon flux rates from 14.4 to 24.0 g of C m^{-2} $year^{-1}$ at depths around 500 m and from 7.2 to 14.4 g of C m^{-2} $year^{-1}$ at seafloor depths (*16*, *29*–*31*). Our calculations of sinker carbon flux are conservative because (i) we undercounted the number of deep sinkers, which are more compact, sink faster, and thus are less likely to be seen; (ii) our sampling was biased toward smaller specimens, because large sinkers did not fit into our samplers; and (iii) we did not count sinkers that had fragmented naturally. Although the measured flux of sinker carbon was variable, the changes did not appear to be closely linked to gross primary production, temperature, or season (*32*) (Fig. 2).

The discarded houses of giant larvaceans thus compose a distinct class of sinking particles that provide a substantial portion of the vertical carbon flux in the deep water column. This is the case off Monterey Bay and probably elsewhere as well. The balance of POC supply and demand measured by Smith and Kaufmann (*9*) at a deep benthic station off central California ranged from occasional surpluses to extended discrepancies of 8 mg of C m^{-2} day^{-1} or more over 7 years. In Monterey Canyon, the daily average of carbon transport by sinking larvacean houses was more than enough to close this gap. Present-day models of carbon flux through the deep water column predict that only ~10% of the POC that sinks below 100 m reaches depths beyond 1000 m (*33*). Our results reveal a pathway through this region that carries substantially more carbon than has been measured by conventional methods. Carbon that reaches the deep sea floor is effectively removed from the atmosphere for geological time scales (*33*).

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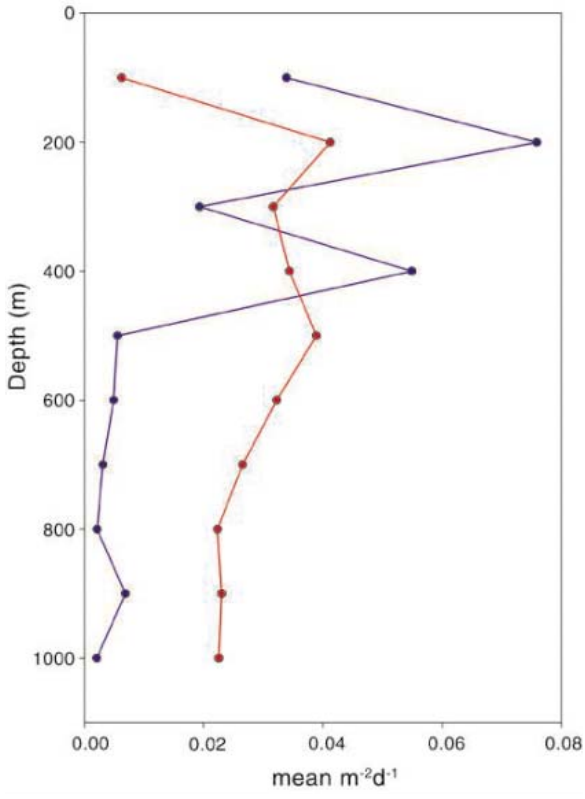

Fig. 3. Comparative plot of active houses of giant larvaceans (blue line) and discarded sinkers (red line) versus depth, in square meters of area swept. The data are derived from a 10-year time series of quantitative video transects at depth intervals between 100 and 1000 m (n = 679 transects). With an average sinking rate of 800 m day^{-1}, the difference between the integrated areas beneath the curves indicates that these animals produce a new house each day (*24*).

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23. Small larvacean species are often very abundant in near-surface waters. Most have bodies less than 10 mm long, with house diameters commonly twice as large. Their houses may be produced at a rate of six or more each day, depending on the density of food particles. Discarded small houses are important components of organic aggregate flux in the ocean's upper layers, but they rarely reach the deep sea floor (*34–36*).
24. Materials and methods are available as supporting material on *Science* Online.
25. Sediment traps catch what they were designed to catch, namely, small, slowly sinking particles. Although sediment traps may occasionally collect sinker fragments, physical contact, particularly with traps that have interior baffles, is certain to exclude, disrupt, or disperse this material (*16*). The easily recognized rectangular mesh structures of larvacean filters have not been reported in analyses of sediment trap contents.
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31. This value considerably exceeds the amount of flux estimated by Silver, Coale, Pilskaln, and Steinberg (*16*), for *Bathochordaeus* in the same region. Although our measurements of the abundance and turnover of houses and sinkers agree, our measurements of the carbon content of sinkers are substantially greater, principally because of incomplete sampling in the earlier study.
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37. We thank the pilots of the ROVs *Ventana* and *Tiburon*, for their skills and patience in the difficult task of collecting these specimens, and the officers and crews of the research vessels *Point Lobos* and *Western Flyer*. Supported by the David and Lucile Packard Foundation.

Supporting Online Material
www.sciencemag.org/cgi/content/full/308/5728/1609/DC1
Materials and Methods
SOM Text
Figs. S1 to S4
References and Notes

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Rapid Acidification of the Ocean During the Paleocene-Eocene Thermal Maximum

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The Paleocene-Eocene thermal maximum (PETM) has been attributed to the rapid release of ~2000 × 10^9 metric tons of carbon in the form of methane. In theory, oxidation and ocean absorption of this carbon should have lowered deep-sea pH, thereby triggering a rapid (<10,000-year) shoaling of the calcite compensation depth (CCD), followed by gradual recovery. Here we present geochemical data from five new South Atlantic deep-sea sections that constrain the timing and extent of massive sea-floor carbonate dissolution coincident with the PETM. The sections, from between 2.7 and 4.8 kilometers water depth, are marked by a prominent clay layer, the character of which indicates that the CCD shoaled rapidly (<10,000 years) by more than 2 kilometers and recovered gradually (>100,000 years). These findings indicate that a large mass of carbon (≫2000 × 10^9 metric tons of carbon) dissolved in the ocean at the Paleocene-Eocene boundary and that permanent sequestration of this carbon occurred through silicate weathering feedback.

During the Paleocene-Eocene thermal maximum (PETM), sea surface temperature (SST) rose by 5°C in the tropics and as much as 9°C at high latitudes (*1–3*), whereas bottom-water temperatures increased by 4° to 5°C (*4*). The initial SST rise was rapid, on the order of ~10^3 years, although the full extent of warming was not reached until some ~30,000 years (30 ky)

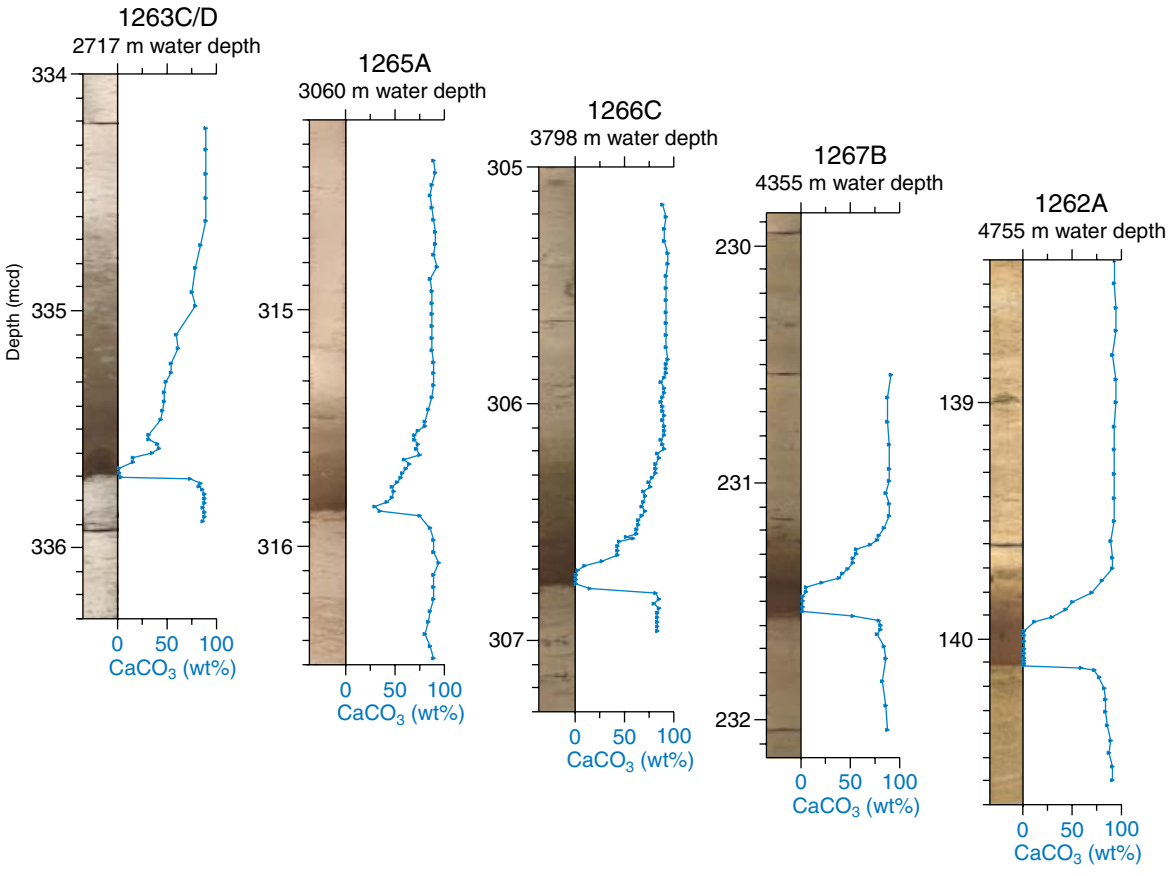

Fig. 1. Digital core photos and weight % $CaCO_3$ content plotted versus meters of composite depth (MCD) across the P-E boundary interval at ODP sites 1262 (hole A), 1263 (hole C/D), 1265 (hole A), 1266 (hole C), and 1267 (hole B) on Walvis Ridge (fig. S1) (*18*). Records are plotted from left to right in order of increasing water depth. The core photos for each site represent composites of the following sections: 1262A-13H-5 and -6; 1263C-14H-1 and core catcher (CC); 1263D-4H-1 and -2; 1265A-29H-6 and -7; 1266C-17H-2, -3, and -4; 1267B-23H-1, -2, and -3.

later (*5*). The most compelling evidence for greenhouse forcing is a coeval global carbon isotope excursion (CIE) of roughly –3.0 per mil (‰) in deep-sea cores (*4*). The pattern of the CIE—an initial rapid decrease (~20 ky) followed by a more gradual recovery (130 to 190 ky) (*1*, *6*–*8*)—indicates the input of a large mass of isotopically depleted carbon into the ocean and atmosphere. Quantitatively, methane hydrates, with a mean $\delta^{13}C$ of <–60‰, appear to be the most plausible source of this carbon (*9*). For example, only ~1200 × 10^9 metric tons of carbon (GtC) of biogenic methane would be required to produce a CIE of 2.5‰ (*10*, *11*). Thermogenic methane has been implicated as well (*12*), although the mass required to produce the CIE would be roughly double that of the biogenic methane.

Regardless of its source, the released methane was rapidly oxidized to CO_2. Subsequent oceanic dissolution of this CO_2 would alter ocean carbon chemistry, principally by lowering the pH and carbonate ion content [CO_3^{2-}] of seawater. These changes would be partially neutralized by a transient rise in the level of the lysocline and calcite compensation depth (CCD) (*13*), resulting in the widespread dissolution of sea-floor carbonate. Eventually, the CO_2 would be sequestered and ocean carbonate chemistry would be restored, primarily through chemical weathering of silicate rocks (*10*). The extent and duration of lysocline/CCD shoaling and subsequent recovery would depend largely on the source, mass, and rate of carbon input. For example, modeling of a 1200-GtC input over 10 ky produces a lysocline shoaling of 300 m (less in the Pacific) with a recovery time of ~40 ky (*10*). Such changes in [CO_3^{2-}] should produce distinct patterns in pelagic carbonate sedimentation and lithology, characterized by an abrupt transition from carbonate-rich sediment to clay, followed by a gradual recovery to carbonate. Moreover, the clay layer should increase in thickness with increasing water depth.

Clay or low-carbonate layers coincident with the PETM were previously identified in several deep-sea cores and land-based marine sections (*14*–*16*). However, these sections, which are either geographically isolated or not completely recovered, or both, are inadequate for constraining CCD variations and for testing the methane hypothesis. Ocean Drilling Program (ODP) Leg 208 was designed to recover an array of pelagic cores spanning the Paleocene-Eocene (P-E) boundary over a broad depth range. The primary drilling target was the Walvis Ridge, in the southeastern Atlantic (fig. S1), where the Deep Sea Drilling Project (DSDP) Leg 74 rotary cored portions of the P-E boundary sequence near the base and summit of the ridge (sites 527 and 525) (*17*). By using advanced piston coring in multiple offset holes at five sites (1262, 1263, 1265, 1266, and 1267), Leg 208 successfully recovered stratigraphically complete and undisturbed upper Paleocene–to–lower Eocene successions at four of five sites between 2.7 and 4.8 km water depth (*18*). At each site, the P-E boundary sequence was characterized by an abrupt transition from carbonate-rich ooze to a dark

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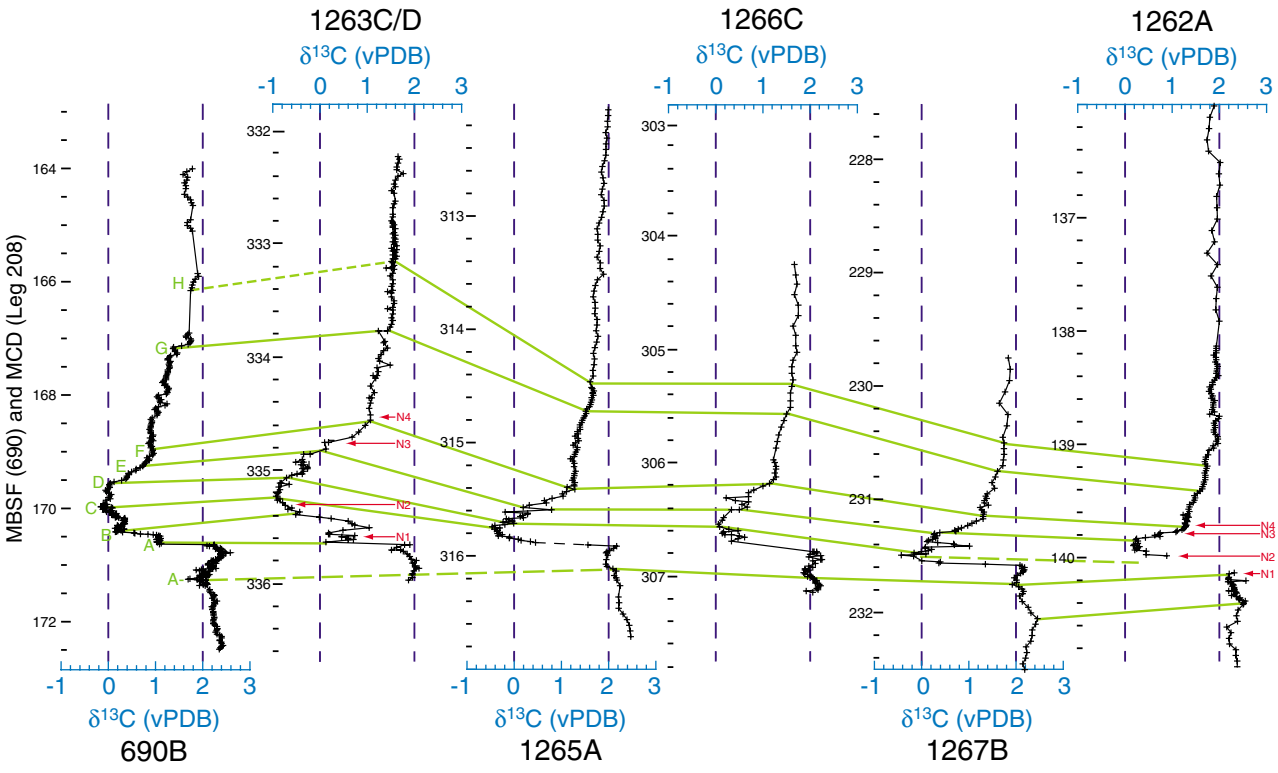

Fig. 2. Bulk sediment carbon isotope records for holes 1262A, 1263C/D, 1265A, 1266C, and 1267B plotted versus MCD. Also plotted are nannofossil horizons (N1 to N4, arrows in red) for holes 1262B and 1263C/D (*20*). Data for ODP site 690 (*22*) are plotted to the far left versus meters below the sea floor (MBSF). Lines of correlation are based on inflections in the carbon isotope (A to G above the P-E boundary, –A below), Fe/Ca, and magnetic susceptibility (MS) records (*20*). vPDB, Vienna PeeDee Belemnite.

red "clay layer," which then graded back into ooze (Fig. 1 and table S1). Carbonate content was <1 weight percent (wt %) in the clay layers, and >80 and 90 wt % in the underlying and overlying oozes, respectively; the only exception was site 1265, where the basal portion of the clay layer was not recovered. The thickness of the clay layers increased with depth, from 5 cm at the shallowest site (1263) [2717 m; paleodepth ~1500 m (*19*)] to 35 cm in the deepest site (1262) [4755 m; paleodepth ~ 3600 m (*19*)] (Fig. 1). The benthic foraminiferal extinction horizon, which is characterized by the disappearance of long-lived Paleocene species and a rapid drop in diversity, occurred at the base of the clay layer in each site (*18*).

Bulk sediment carbon isotope ($\delta^{13}C$) records were constructed at 1- to 5-cm resolution for each boundary section (table S2) (*20*). Each record is marked by a decrease in $\delta^{13}C$ at the base of the clay layer, followed by gradual recovery. Minimum carbon isotope values within the clay layer are not uniform, but increase from the shallowest to the deepest site (minimums of –0.9 and 0.0‰ at sites 1263 and 1262, respectively), a feature we attribute to truncation by dissolution and the presence of residual pre-excursion calcite (*21*). Also, the base of the CIE differs across sites, occurring in two steps at site 1263 and in a single step at the deeper sites. As a result, the excursion layer, from the onset of the CIE to the point of full recovery (i.e., stability), decreases in thickness from 2.1 m at site 1263 to 1.0 m at 1262.

In this spatially tight array of sites, the production and export of carbonate and the accumulation of clay should be similar at any given time, leaving dissolution as the major process that drives differences in carbonate accumulation between sites. We can therefore infer from the weight % carbonate and carbon isotope data that rapid shoaling of the lysocline/CCD occurred, followed first by a more gradual descent or recovery of the CCD and then by the recovery of the lysocline. The duration of the lysocline/CCD descent from the shallowest to the deepest sites was estimated by first correlating several key inflection points in the carbon isotope records (Fig. 2, tie points A to G), as well as in the Fe concentration and bulk magnetic susceptibility (MS) records (fig. S2). The tie points, particularly E and F, were then verified with biostratigraphic data (table S3) (*20*). We then correlated the site 1263 carbon isotope record to that of south Atlantic ODP site 690 (*22*), which has an orbitally derived age model (*8*), and ordinated the weight % carbonate and isotope data for each site within that age model (Fig. 3 and table S4). An alternate age model based on ^3He exists for site 690 (*23*), but the two models are roughly similar for the initial 100 ky of the PETM; thus, the choice of model makes little difference in our interpretation of events up to that point. The greatest uncertainty in the site-to-site correlations and age estimates is in the basal portion of the clay layer, where the carbon isotope and other records are compromised by dissolution. The correlations (Fig. 1, tie points D to G) are most reliable in the recovery interval where the weight % carbonate is higher and the ocean $\delta^{13}C$ is rapidly shifting.

Given these age constraints, the CCD is inferred to have shoaled more than 2 km within a few thousand years (Fig. 3). Recovery was gradual, with the CCD descending to the shallowest site (1263) within ~10 to 15 ky of the CIE onset and to the deepest site (1262) within ~60 ky. By +110 ky, carbonate content had fully recovered. This pattern of change, particularly the recovery, has important implications. According to theory, the initial uptake of CO_2 and buffering should occur mainly via deep-sea calcite dissolution, but eventually, chemical weathering of silicate rocks takes over accelerating the flux of dissolved ions (including HCO_3^-) to the ocean, thereby increasing [CO_3^{2-}] and the rate of calcite accumulation (*24*). The distribution of carbonate between +60 and +100 ky indicates that the CCD had descended, but the lysocline was still shallow and the deep sea was largely undersaturated. The percentage of $CaCO_3$ continued to increase, and by +110 kyr, it had reached 90% over the entire

transect, a state that implies that the lysocline descended below the deepest site (>3.6 km) as well as its pre-excursion level. This phenomenon is consistent with theory (*10*) and likely represents a transitional period during which the excess ions supplied to the ocean by the weathering of silicate rocks greatly increased deep-sea CO_3^{2-} concentration and thus carbonate accumulation. The site 690 record is marked by a similar pronounced interval of high carbonate content (*23*, *25*), demonstrating that CO_3^{2-} oversaturation was not a local phenomenon.

This scenario for acidification of the deep sea and initial neutralization by calcite dissolution is not unlike that simulated by models in response to the anthropogenic rise in CO_2 (*26*–*28*). Because dissolution layers are also present in P-E boundary sections in the Pacific and Tethys Oceans and at depths <1 km (*29*–*33*), it appears that for a brief period of time, much of the ocean beneath the thermocline was highly undersaturated with respect to calcite. The mass of CO_2 required to shoal the CCD to <1 km water depth would be substantial. In a series of simulations with an ocean/sediment carbon-cycle model designed to evaluate the ocean-buffering capacity in response to a range of anthropogenic CO_2 fluxes, 4500 GtC was required to terminate carbonate accumulation over the entire ocean (*26*).

For the PETM, the release of >4500 GtC would be more consistent with the magnitude of global temperature rise (*2*, *3*, *9*). Such a large mass of carbon, however, would require a reevaluation of the source of carbon and its isotopic composition. With bacterially produced methane at –60‰, the total input from hydrates is limited by the $\delta^{13}C$ excursion to <2000 GtC (*10*). To increase the mass of carbon added while adhering to the isotope constraints requires the input of isotopically heavier carbon, such as thermogenic CH_4/CO_2 (~–30 to –20‰) or oxidation of organic carbon (standing or stored) (–20‰) (*34*). In this regard, recent documentation of an unusual concentration of upper Paleocene fluid/gas seep conduits associated with volcanic intrusions in the North Atlantic (*12*) merits additional attention. An alternative explanation, that the magnitude of the marine CIE has been greatly underestimated because of dissolution or damping by pH affects, seems unlikely given the constraints provided by continental isotope records (*35*). Finally, proximity to where carbon (CO_2 or CH_4) enters the deep sea via circulation will dictate where neutralization by carbonate dissolution is most intense (*36*). For example, severe dissolution in the Atlantic may indicate direct input of methane into bottom waters entering this basin.

Excessive carbonate undersaturation of the deep ocean would likely impede calcification by marine organisms and therefore is a potential contributing factor to the mass extinction of benthic foraminifera at the P-E boundary. Although most plankton species survived, carbonate ion changes in the surface ocean might have contributed to the brief appearance of weakly calcified planktonic foraminifera (*6*) and the dominance of heavily calcified forms of calcareous algae (*37*). What, if any, implications might this have for the future? If combustion of the entire fossil fuel reservoir (~4500 GtC) is assumed, the impacts on deep-sea pH and biota will likely be similar to those in the PETM. However, because the anthropogenic carbon input will occur within just 300 years, which is less than the mixing time of the ocean (*38*), the impacts on surface ocean pH and biota will probably be more severe.

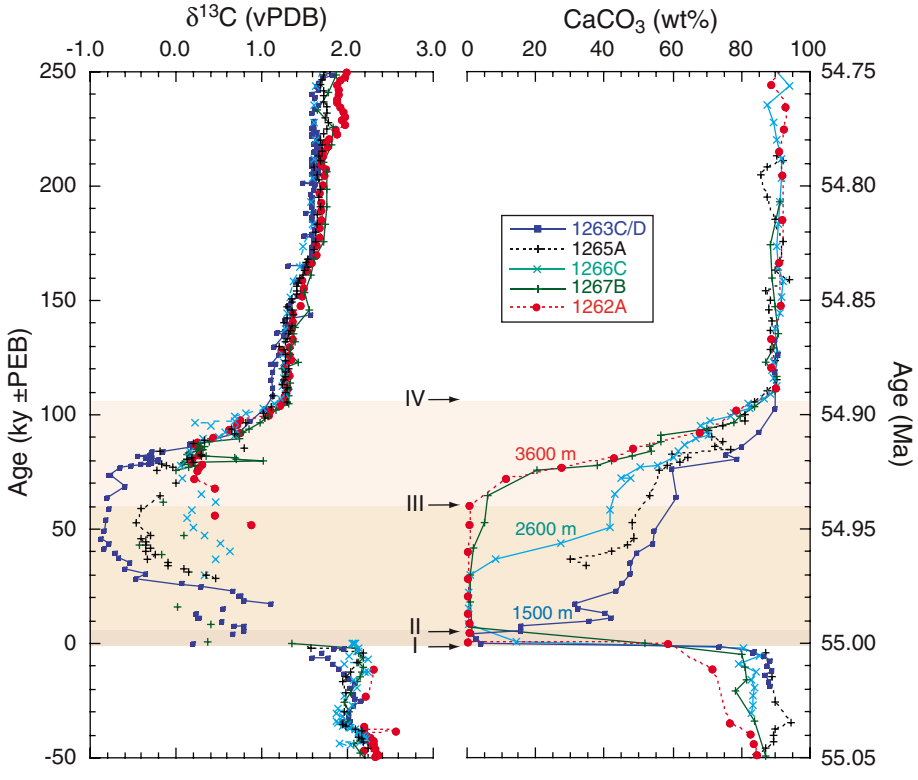

Fig. 3. Bulk sediment $\delta^{13}C$ and weight % carbonate content ($g_{CaCO3}/g_{Total} \times 100$) plotted versus age for ODP sites 1262, 1263, 1265, 1266, and 1267. Age (ky) relative to the P-E boundary is plotted on the left axis and absolute age (Ma) along the right. Age models (table S4) are based on correlation to site 690 (*8*) using the carbon isotope stratigraphy as verified with the nannofossil events in Fig. 2 and with the Fe and MS cycles in fig. S2. Transferring the 1263 age model to deeper sites with carbon isotopes could only be achieved where sufficient carbonate was present. Ages within the clay layers for sites 1266, 1267, and 1262 were derived through linear interpolation from tie points E and A. Paleodepths (~55 Ma) are provided for sites 1263 (1500 m), 1266 (2600 m), and 1262 (3600 m). Key events in the evolution of south Atlantic carbonate chemistry were (i) the rapid drop in content to <1% for all sites with the exception of site 1265, where the lowermost Eocene is absent (marked I); (ii) the return of the CCD to site 1263 roughly 5 ky after the excursion (marked II); (iii) the return of the CCD to site 1262 at 60 ky (marked III); and (iv) the lysocline descending to a point below the deepest site at 110 ky after the excursion (marked IV). PEB, Paleocene-Eocene boundary.

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13. The lysocline, also referred to as the calcite saturation horizon, represents the depth in the ocean where the carbonate ion concentration falls below the saturation level (currently ~4 km in the South Atlantic). Carbonate accumulation can occur below this level if the flux of carbonate to the sea floor exceeds the rate at which it dissolves. The depth at which dissolution is greater than the flux and where carbonate does not accumulate defines the CCD.
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19. Paleodepths of the Leg 208 sites at 55 million years ago (Ma) were estimated using a standard thermal subsidence curve and a sediment accumulation model (*18*). At 55 Ma, the paleodepths of sites 1263 and 1262 were 1.5 and 3.6 km, respectively.
20. Materials and methods are available as supporting material on *Science* Online.
21. The initial phase of dissolution would involve Paleocene sediments already present on the sea floor. As such, the base of the clay layer, perhaps as much as a few centimeters in the deepest site, was deposited before the carbon isotope excursion. The traces of carbonate remaining must be a mixture of pre-excursion and excursion fragments that survived dissolution.
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24. The weathering of silicates on land is generally represented by the following equation: $CaSiO_3 + 2CO_2 + H_2O \rightarrow 2HCO_3^- + Ca^{2+} + SiO_2$. Ensuing precipitation of calcite from the bicarbonate (and carbonate) ions supplied by the above reaction is represented by this equation: $HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + CO_2 + H_2O$, so that there is a net uptake of one unit of CO_2 for each unit of silicate weathered.
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38. The primary buffering capacity of the ocean is provided by the deep ocean and sea-floor sediments. Because the mixing time of the ocean is >500 years, most of the anthropogenic CO_2 will accumulate in the atmosphere and surface ocean before it can be conveyed to the deep sea to be neutralized (*27*).
39. We thank the ODP Leg 208 Science Crew for their contributions and C. John and S. Bohaty for technical assistance. The ODP supplied samples. Supported by NSF grant EAR-0120727 to J.C.Z. and E.T. and by DFG grant no. Ro 1113/3 to U.R.

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Photoinduced Plasticity in Cross-Linked Polymers

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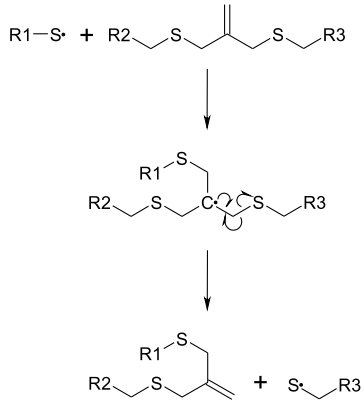
Chemically cross-linked polymers are inherently limited by stresses that are introduced by post-gelation volume changes during polymerization. It is also difficult to change a cross-linked polymer's shape without a corresponding loss of material properties or substantial stress development. We demonstrate a cross-linked polymer that, upon exposure to light, exhibits stress and/or strain relaxation without any concomitant change in material properties. This result is achieved by introducing radicals via photocleavage of residual photoinitiator in the polymer matrix, which then diffuse via addition-fragmentation chain transfer of midchain functional groups. These processes lead to photoinduced plasticity, actuation, and equilibrium shape changes without residual stress. Such polymeric materials are critical to the development of microdevices, biomaterials, and polymeric coatings.

Cross-linked, gelled polymers have an "infinite" molecular weight and are described as thermosets, implying a network that cannot be melted or molded (*1*). This description is true for most chemically cross-linked polymers; however, several cross-linked networks are known to undergo bond cleavage or depolymerization at high temperatures or under various chemical or other treatments (*2*). Although such treatments are useful for recycling purposes, there is an associated degradation in the mechanical properties of the polymers. "Crack-healing" networks, such as those that use groups in the polymer backbone able to undergo thermoreversible Diels-Alder reactions (*3*), are able to relieve stress without mechanical degradation. However, this reaction must be performed at elevated temperatures, making it unsuitable in thermally sensitive applications such as dental composites. Internal stress buildup during polymerization is typical when shrinkage occurs. This stress decreases the ultimate mechanical properties of the cured polymer, which is highly detrimental in fields such as polymeric coatings, fiber-reinforced composites, and dental materials, or it may introduce birefringence, unwanted in optical materials. Additionally, given that the equilibrium shape of conventional cross-linked polymers is defined by the shape at gelation, stress relief would enable a material to be "molded" and subsequently destressed, allowing for arbitrary equilibrium shapes to be attained after cure.

We describe a covalently cross-linked network that is able to undergo photomediated, reversible cleavage of its backbone to allow chain rearrangement for rapid stress relief at ambient conditions without mechanical property degradation. The key to this reversible backbone cleavage is addition-fragmentation chain transfer. Reaction diffusion of radicals through the cross-linked matrix occurs initially by reaction of a radical with an in-chain functionality, forming an intermediate, which in turn fragments, reforming the initial functionality and radical. Allyl sulfides have been used as efficient addition-fragmentation chain transfer agents (*4*–*6*). This addition-fragmentation process alters the topology of the network, but the polymer chemistry and network connectivity remain unchanged. In the absence of radical termination events or other side reactions, the number of allyl sulfide groups, and hence network strands, remains unchanged (Scheme 1), although relaxation of the stresses in each bond is facilitated by the alternating cleavage and reformation reactions.

The monomers used to produce the networks are shown in Scheme 2. The base network studied was formed from a stoichiometric mixture of pentaerythritol tetra(3-mercaptopropionate) (PETMP) and triethyleneglycol divinylether (TEGDVE), which produces a rubbery network with a glass transition temperature (T_g) of about –25°C. This monomer system was modified by the addition of varying concentrations of the ring-opening monomer 2-methyl-7-methylene-1,5-dithiacyclooctane (MDTO) (*7*) as a comonomer. Addition of a stoi-



Scheme 1. Reaction mechanism for chain transfer within the polymer backbone.

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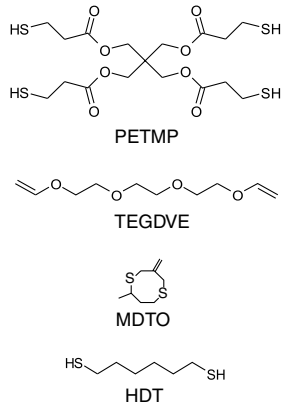

Scheme 2. Monomers used to produce the networks.

chiometric amount of 1,6-hexanedithiol (HDT) and TEGDVE to the tetrathiol/divinylether was used to produce an alternative traditional network with a lower cross-link density and rubbery modulus. Typically, thiol-ene polymerizations follow a step-growth radical mechanism (*8*) with alternating thiol and ene monomer units. Addition of the ring-opening monomer does not alter the stoichiometry of this network, because the carbon-centered vinyl ether radical only abstracts a hydrogen from the thiol and the ring-opening monomer propagates via a sulfur-centered radical (*7*, *9*–*11*). Thus, whereas ring-opening monomers are more typically used to reduce the shrinkage due to polymerization (*12*), we used MDTO to conveniently introduce the reversibly cleavable allyl sulfide functionality regularly throughout the polymer backbone.

Confirmation of the cross-linked nature of fully cured specimens was obtained by dynamic mechanical analysis, in which a specimen is sinusoidally deformed during a temperature ramp to determine the real (storage) and imaginary (loss) components of the modulus. The value of T_g was systematically reduced as the concentration of ring-opening monomer was increased [T_g = –34°C for 75 weight percent (wt %) MDTO] as a result of the reduced cross-link density; however, all specimens displayed a rubbery plateau modulus typical of cross-linked polymers. All the specimens were clearly within the rubbery regime at ambient temperature. Additionally, although all the specimens were readily swollen with common organic solvents, none were soluble—again a defining feature of cross-linked polymers.

Strain profiles of the specimens during and after irradiation are presented in Fig. 1. During irradiation, homolytic photolysis of residual photoinitiator produces radicals in the specimens. Diffusion of these radicals occurs via addition-fragmentation chain transfer through the allyl sulfide functionalities. As a result, the polymer backbone is repeatedly cleaved, stress is alleviated, and the backbone is reformed in a less stressed conformation. Neither of the neat thiol-ene specimens contain any groups in the backbone capable of this cleavage; thus, the small amount of deformation that occurs upon irradiation is primarily due to the minimal heating induced by the light exposure. The sequential cleavage and reformation in the remaining networks repeats as long as radicals are produced, because the addition-fragmentation chain transfer reaction does not consume functional groups. The ductility or malleability of these cross-linked networks is limited only by the radical generation and termination reactions.

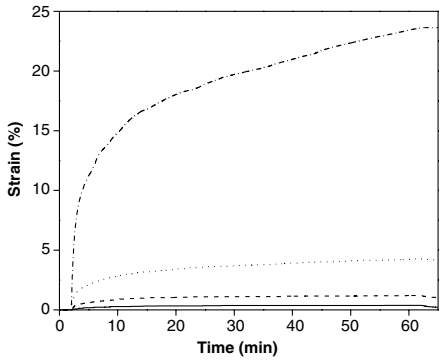

Fig. 1. Strain profiles of tetrathiol/divinyl ether specimens with varying concentrations of MDTO (solid line, 0 wt %; dashed line, 25 wt %; dotted line, 50 wt %; dashed-dotted line, 75 wt %). The specimens were under tensile stress of ~10^5 Pa throughout the experiment and were irradiated (320 to 500 nm, 30 mW cm^{-2}) from t = 2 min to t = 62 min.

The variation in the degree of strain for specimens with differing MDTO concentrations (Fig. 1) is due to the increasing concentration of allyl sulfide groups in the network strands. At elevated MDTO concentrations, the concentration of allyl sulfide groups in the network increases, and there is a small, corresponding increase in the length of the average network strand and a decrease in the cross-link density (Table 1). Consequently, the rate at which strands are broken and stress is relieved is substantially higher at elevated MDTO concentrations.

The reversible strain in the neat thiol-ene polymer networks (Fig. 1) was attributed to a small thermal expansion effect due to heating during irradiation. Temperature rise measurements indicated an increase in temperature of ~5°C during the first minute; the temperature then remained at 5°C above room temperature for the remainder of the irradiation. The measured temperature rise profile was unaffected by the specimen composition. The apparent strain recovery of the neat thiol-ene polymer networks upon cessation of irradiation was due to the specimens cooling in the dark.

In the absence of radiation, upon application of stress, each specimen underwent a degree of strain that was dependent on the modulus and therefore the concentration of MDTO in the specimen. When the stress was released, the specimen returned to its original length. This situation was altered when the specimens were irradiated during the stress application. The reversible and irreversible components of the strain during the application of stress and irradiation are readily observed in Fig. 2. The strain is completely reversible when a cyclic stress is applied without irradiation (Fig. 2A); however, an irreversible component is clearly observed when irradiation is applied at the same time as the stress (Fig. 2B) for all samples that contain MDTO. As shown in Fig. 2 for both the lower and higher modulus control materials that do not contain MDTO, no alteration of the equilibrium strain is possible in the absence of the allyl sulfide linkages.

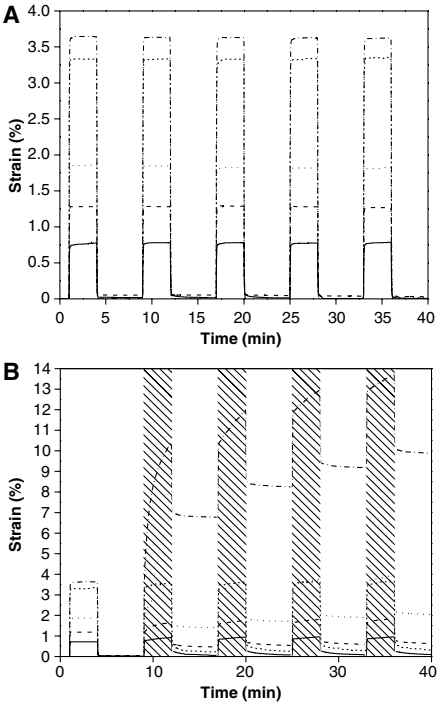

Fig. 2. Strain/recovery profiles of tetrathiol/divinyl ether specimens with varying concentrations of MDTO (solid line, 0 wt %; dashed line, 25 wt %; dotted line, 50 wt %; dashed-dotted line, 75 wt %) and a 25 wt % tetrathiol/divinyl ether–75 wt% dithiol/divinyl ether specimen (short-dashed line). The stress was alternated between 0 and 10^5 Pa. (**A**) Without irradiation. (**B**) Irradiation (320 to 500 nm, 30 mW cm^{-2}) during stress application after the first load cycle (irradiation indicated by the shaded areas).

Stress relaxation experiments were performed by deforming the specimens to a certain strain and subsequently irradiating them. The results (Fig. 3A) directly show the relaxation of stress in the specimens during irradiation. A small decrease in the stress experienced in the unmodified thiol-ene specimens was again attributed to thermal expansion due to heating from the lamp; however, the specimens containing the allyl sulfide groups all show substantial stress relaxation.

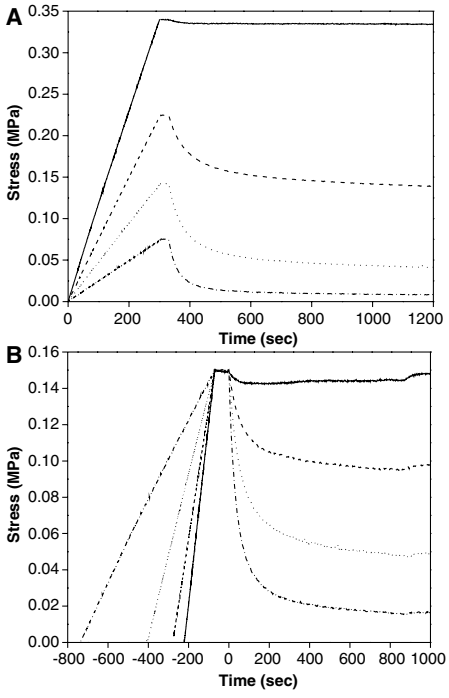

Fig. 3. Stress versus time for four MDTO concentrations (solid line, 0 wt %; dashed line, 25 wt %; dotted line, 50 wt %; dashed-dotted line, 75 wt %). (**A**) Constant strain (irradiation started at $t = 330$ s). (**B**) Constant initial stress (offset to align the start of irradiation at $t = 0$, irradiation stopped at 900 s). The specimens were irradiated at 320 to 500 nm, 20 mW cm^{-2}.

Table 1. Tensile moduli of specimens before and after experiments performed in Fig. 3A.

MDTO (wt%)	Ratio of cross-links to allyl sulfide groups	Modulus before extension and irradiation (MPa)	Modulus after extension and irradiation (MPa)
0	1: 0	11.5	11.8
25	1.17: 1	7.33	7.72
50	0.390: 1	4.58	5.17
75	0.130: 1	2.38	2.92
0 (75 wt % dithiol/divinyl ether)	1: 0	3.21	3.38

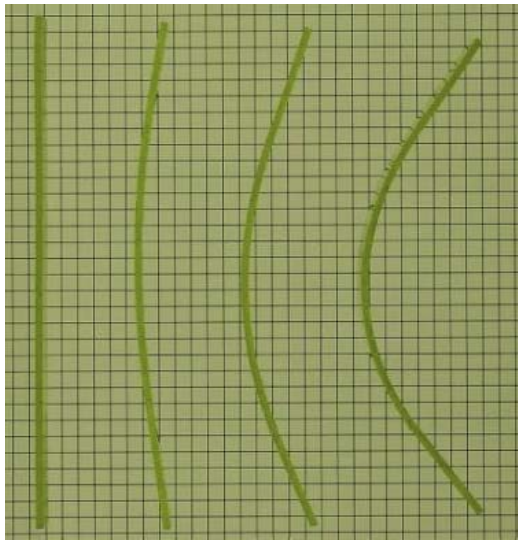

Fig. 4. Specimens with stress gradients through their thickness on a 2 mm by 2 mm grid. Specimens from left to right: 0, 25, 50, and 75 wt % MDTO. The direction of irradiation (365 nm, 20 mW cm^{-2} for 15 s) used for the creation of the stress gradient for each specimen was from left to right.

The stress relaxation is accompanied by a variation in the specimen dimensions when the specimens are removed from the instrument clamps (greater than 1 mm lengthwise for the 75 wt% MDTO specimen in Fig. 3A), demonstrating the induced plasticity via the introduction of radicals in the system.

A clearer picture of the effect of the MDTO concentration on the rate and degree of stress relaxation is seen in Fig. 3B. At a constant applied stress, both the rate and degree of stress relaxation increase with MDTO concentration. We again attribute this response to an increased probability of addition-fragmentation chain transfer groups in the network strands. After cessation of irradiation, the stress actually rises slightly because of specimen shrinkage upon cooling.

If the stress relaxation had been the result of photodegradation, the results in Figs. 1 and 3 would have been similar. It is possible to determine whether the network is simply undergoing photodegradation during irradiation by measuring the modulus of the material before and after irradiation. The elastic moduli (determined in tension) of the specimens before and after the irradiation experiments shown in Fig. 3A are presented in Table 1. The slight increase in the modulus after irradiation clearly indicates that photodegradation is not responsible for the stress relaxation. Additionally, the results in Fig. 2 would appear different if photodegradation were responsible for the observed behavior, because the magnitude of the reversible strain would vary with irradiation; however, such variation is not observed. Fourier transform infrared spectroscopy analysis of the allyl sulfide linkages in the polymer also shows no measurable net degradation of these groups as a result of irradiation.

One of the many interesting applications of this phenomenon is the deliberate introduction of stress gradients in a cross-linked material. Irradiation of an optically dense specimen under stress leads to the release of stress only on the exposed side. As a result, once the stress is released, the specimen warps away from the direction of irradiation (Fig. 4). This curvature is released by irradiation of the previously unexposed side. As a result, a shape-change or actuation phenomenon may be effected without the typical increase in temperature required by shape-memory polymers (*13*, *14*).

Although our study involved model rubbery networks, this process may also be applied to a vast array of other applications for which the control or elimination of stress is critical. Specifically, it is feasible to produce low residual stress in high-T_g materials by incorporating this relaxation process throughout the curing reaction. Groups capable of undergoing these chain transfer reactions need not be introduced via the copolymerization of a ring-opening monomer as performed in this work. Rather, cross-linking monomers (e.g., diacrylates, dimethacrylates) can be envisaged that contain linear addition-fragmentation functionalities incorporated in the monomer structure between the multiple polymerizable functionalities. Thus, networks with high cross-link density and high T_g that contain the relevant functionalities may be readily synthesized, with the concomitant benefit of reduced stress resulting from the polymerization.

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Protection from Experimental Asthma by an Endogenous Bronchodilator

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Mechanisms that protect against asthma remain poorly understood. *S*-nitrosoglutathione (GSNO), an endogenous bronchodilator, is depleted from asthmatic airways, suggesting a protective role. We report that, following allergen challenge, wild-type mice exhibiting airway hyperresponsivity have increased airway levels of the enzyme GSNO reductase (GSNOR) and are depleted of lung *S*-nitrosothiols (SNOs). In contrast, mice with genetic deletion of GSNOR exhibit increases in lung SNOs and are protected from airway hyperresponsivity. Our results indicate that endogenous SNOs, governed by GSNOR, are critical regulators of airway responsivity and may provide new therapeutic approaches to asthma.

The current understanding of allergic asthma, characterized by airway hyperresponsivity (AHR) and chronic airway inflammation, is centered on the role of bronchoconstrictor substances and inflammatory mediators (*1*–*3*). The role of endogenous airway relaxants in the pathogenesis of asthma has received less attention, and the relative importance of impaired airway relaxation versus active constriction is unknown.

Nitric oxide (NO) has been implicated in the regulation of airway tone (*4*), and elevated levels of NO in the exhaled breath are a signature of asthma (*5*, *6*), attributed in part to up-regulation of cytokine-inducible NO synthase (iNOS) within the airways (*7*–*10*). However, neither genetic deletion of iNOS in mice nor pharmacological inhibition of NOS in asthmatic patients has provided significant protection against AHR (*7*, *10*, *11*). Furthermore, animals deficient in NO synthases that are expressed constitutively in the lung (eNOS, nNOS, and iNOS) do not exhibit increases in airway tone or AHR (*7*).

Accumulating evidence indicates that NO bioactivity is conveyed largely through the covalent modification of cysteine sulfurs by NO to form *S*-nitrosothiols (SNOs) (*12*–*14*). Of these, *S*-nitrosoglutathione (GSNO) represents a major source of bronchodilatory NO bioactivity (airway concentrations of GSNO are much higher than those of NO) (*15*), and abnormal metabolism of GSNO (reflecting altered NOS activity) has been described in several lung diseases (*15*–*17*). Paradoxically, although NOS activity is increased in human asthma, it has been reported that GSNO is depleted from airway lining fluid (ALF) (*18*, *19*); the functional consequences of this disequilibrium are not currently understood. We have recently described an enzyme, GSNO reductase (GSNOR), which governs levels of GSNO [and, indirectly, protein SNO (*20*)] and which is expressed widely across tissues, including the lung (*12*, *20*). We used mice with targeted deletion of the GSNOR gene ($GSNOR^{-/-}$) (*21*) to elucidate the role of SNOs in the regulation of airway tone and pathogenesis of asthma.

GSNOR activity was first measured in ALF. In wild-type (WT) mice, GSNOR activity was absent from ALF at basal conditions [after phosphate-buffered saline (PBS) treatment] but was readily detected after exposure to the allergen ovalbumin (OVA) (*21*) (Fig. 1A). As expected, GSNOR activity was not detected in ALF from $GSNOR^{-/-}$ mice under either condition (*22*). Immunohistochemistry revealed that GSNOR was present in multiple cell types in control and OVA-challenged WT lungs, including airway epithelial cells and infiltrating leukocytes (fig. S1). The presence of GSNOR in ALF after OVA challenge may thus reflect release as a consequence of epithelial damage or lysis of inflammatory cells, as described in asthmatic patients (*3*, *8*).

SNO levels were next assayed in homogenates of lung tissue from $GSNOR^{-/-}$ and WT mice. Under basal conditions (PBS treatment), SNO proteins could be detected but only at the limits of sensitivity of the assay used (*22*, *23*). However, after OVA treatment SNO levels were easily quantified (*12*, *21*) and were substantially elevated in $GSNOR^{-/-}$ as compared with WT lungs (Fig. 1B). These results indicate that endogenous SNOs are metabolized by GSNOR under asthmatic-like conditions.

To determine whether the effects of GSNOR deletion were independent of NO generation, levels of nitrite and nitrate (i.e., NO metabolites) were measured in ALF after OVA or PBS treatment. In both $GSNOR^{-/-}$ and WT mice, nitrite concentrations increased after exposure to OVA, whereas nitrate concentrations were unaffected (Fig. 1C). In contrast to SNOs, however, nitrite and nitrate concentrations did not differ significantly in $GSNOR^{-/-}$ versus WT mice (Fig. 1C). In addition, Western blot analysis of lung lysates showed that iNOS expression was comparable in $GSNOR^{-/-}$ and WT mice, both at baseline and after OVA challenge (*22*). Taken together, these data indicate that the dysregulation of SNO homeostasis in the lungs of $GSNOR^{-/-}$ mice was independent of NOS activity.

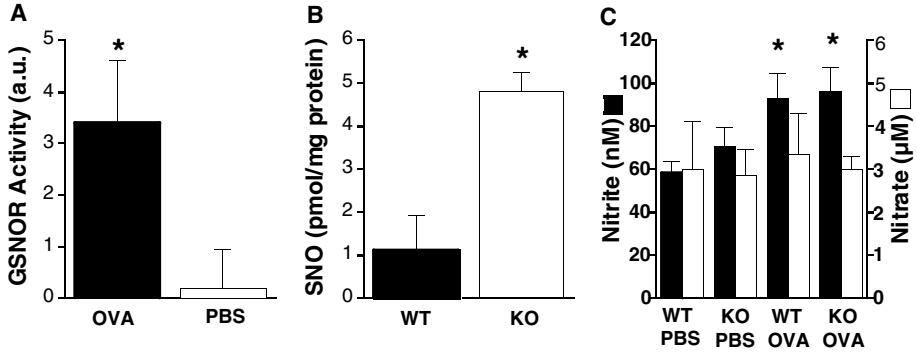

Fig. 1. (**A**) GSNOR activity in bronchoalveolar ALF (cell-free) of WT mice is increased in response to OVA sensitization and challenge. Data represent the mean + SE of samples from at least seven OVA-treated and control (PBS) mice (*, $P < 0.05$; Student's two-tailed *t* test). (**B**) Levels of *S*-nitrosylated proteins (SNO) in lung homogenates of $GSNOR^{-/-}$ mice [assayed as described in (*12*)] were significantly higher than in WT controls after OVA treatment. Data were normalized for protein content of total lysates and represent the mean + SE of at least three WT and $GSNOR^{-/-}$ (KO) mice (*, $P < 0.02$; Student's two-tailed *t* test). (**C**) Levels of ALF nitrite (filled) and nitrate (open) were similar in WT and $GSNOR^{-/-}$ mice after either control (PBS) or allergen (OVA) treatment. An asterisk indicates significant pairwise differences between OVA-treated and control mice ($P < 0.04$, $n = 12$ to 20). Means + SE are shown.

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The influence of GSNOR in the regulation of airway tone and development of AHR was next assessed with a Flexivent system in a standard model of allergen-induced asthma (*21*) (Fig. 2). At baseline (PBS treatment), total pulmonary resistance (R_T), which provides a measure of airway tone, was significantly lower in GSNOR$^{-/-}$ than in WT mice (1.5 versus 1.8 cm H_2O/ml/s; $n = 14$ per group, $P = 0.001$), and the airway response to the bronchoconstrictor agonist methacholine (MCh) was significantly lower in PBS-treated GSNOR$^{-/-}$ mice versus WT controls (Fig. 2A; $P < 0.001$). At high MCh doses, the MCh-induced airway resistance was approximately two-thirds lower in GSNOR$^{-/-}$ than in WT mice (Fig. 2A). R_T did not differ at baseline (i.e., premethacholine) after OVA treatment [$n = 16$ per group (*22*)]. More important, whereas OVA treatment in WT mice resulted in a marked increase in airway responsiveness (i.e., AHR), OVA-treated GSNOR$^{-/-}$ mice showed little increase in airway responsivity (Fig. 2, B and C; $P < 0.004$ versus WT), demonstrating that GSNOR$^{-/-}$ mice are protected in this asthma model. These data indicate that GSNOR regulates basal airway tone, as well as airway responsiveness to both bronchoconstrictor agonists and allergen challenge.

To further verify the role of GSNO in the control of airway reactivity and to discriminate its effects from those of NO, WT and GSNOR$^{-/-}$ animals were treated with 1400W, a selective iNOS inhibitor (*12*). In OVA-treated WT mice, inhibition of iNOS did not significantly alter airway responsiveness (Fig. 2D). In contrast, OVA-treated GSNOR$^{-/-}$ mice displayed markedly heightened airway responsivity after iNOS inhibition (Fig. 2E; $P < 0.02$), accompanied by a commensurate reduction in SNO levels (Fig. 2F; $P < 0.05$). Indeed, after iNOS inhibition, neither the levels of SNO nor airway responsivity differed between OVA-treated GSNOR$^{-/-}$ and WT mice (Fig. 2, D, E, and F). iNOS inhibition (1400W) had little effect, however, on the airway responses of PBS-treated control animals (e.g., MCh response in PBS-treated GSNOR$^{-/-}$ mice; Fig. 2E), suggesting that basal airway tone is regulated by eNOS or nNOS. Collectively, these findings indicate that GSNOR$^{-/-}$ mice are protected from allergen challenge and that GSNO activity, originating from iNOS, serves to promote bronchodilation under asthmatic-like conditions. In addition, the genetic evidence suggests a role for GSNO, derived from eNOS or nNOS, in the regulation of basal airway tone.

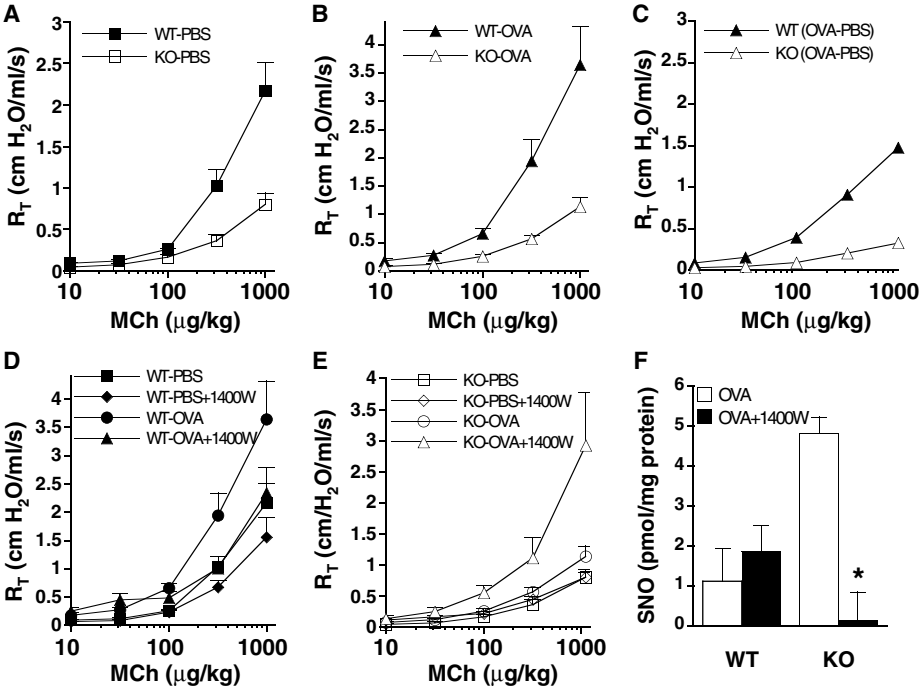

Fig. 2. Airways of GSNOR$^{-/-}$ mice are hyporeactive to methacholine (MCh) and allergen challenge. Total pulmonary resistances (R_T) of WT and GSNOR$^{-/-}$ (KO) mice after control (nonallergic; PBS) (**A**) and allergen (OVA) (**B**) treatment were determined in the absence or presence of various concentrations of MCh administered intravenously. R_T values in PBS-treated and in OVA-treated GSNOR$^{-/-}$ mice were significantly lower than in WT controls [KO PBS versus WT PBS, $P < 0.001$; KO OVA versus WT OVA, $P < 0.004$; analysis of variance (ANOVA) and post-hoc analyses at 3- to 5-MCh doses]. Data represent the mean + SE of at least 7 to 10 mice per group. (**C**) The incremental effect of OVA (over PBS control) on WT and GSNOR$^{-/-}$ mice [OVA minus PBS (OVA-PBS)]. Whereas R_T of WT mice increased significantly after OVA treatment (WT PBS versus WT OVA, $P < 0.04$; ANOVA), the R_T of GSNOR$^{-/-}$ mice did not change significantly (KO PBS versus KO OVA, $P = 0.1$; ANOVA). (**D**) Effect of the iNOS inhibitor 1400W on airway responsiveness in PBS- and OVA-treated WT mice (WT PBS versus WT PBS + 1400W, $P = 0.12$, $n = 5$ to 9; WT OVA versus WT OVA + 1400W, $P = 0.22$, $n = 7$ to 9). (**E**) Effect of iNOS inhibition by 1400W on airway responsiveness in GSNOR$^{-/-}$ mice. Administration of 1400W to OVA-treated GSNOR$^{-/-}$ mice resulted in a significant increase in airway resistance (KO OVA versus KO OVA + 1400W, $P < 0.02$, $n = 5$ to 9; ANOVA). (**F**) Protein *S*-nitrosylation (SNO) in lung homogenates of OVA-treated mice. iNOS inhibition (1400W) reduces SNO levels in GSNOR$^{-/-}$ mice (*, $P < 0.05$, $n = 3$).

To investigate potential cellular mechanisms by which GSNO regulates airway reactivity, we performed a bioassay (*21*) that measured the tone of tracheal rings obtained from WT and GSNOR$^{-/-}$ mice (Fig. 3). Tracheal rings from GSNOR$^{-/-}$ and WT mice contracted similarly when stimulated by carbachol, a parasympathetic agonist (Fig. 3A), and relaxed similarly to GSNO (Fig. 3B). Thus, the decreased airway response to MCh in GSNOR$^{-/-}$ mice in vivo (Fig. 2) is unlikely to have resulted from direct effects on cholinergic receptor–mediated activity. In contrast, tracheal relaxation in response to the β-adrenergic agonist, isoproterenol, differed between GSNOR$^{-/-}$ and WT mice. Specifically, tracheal rings of WT mice desensitized upon repeated exposure to isoproterenol, whereas relaxations of tracheal rings from GSNOR$^{-/-}$ mice were maintained (Fig. 3, C and D). Furthermore, the desensitization of WT tracheal rings was prevented by pretreatment with GSNO (Fig. 3, E and F). Thus, abundance of GSNO evidently represents an important determinant of airway responsivity to β-adrenergic agonists. Interestingly, a recent study demonstrated that vascular tachyphylaxis induced by isoproterenol when NOS is inhibited can be prevented by exogenous *S*-nitrosothiols (*24*). Taken together, these data suggest that GSNO levels, governed by the catalytic activity of GSNOR, can modulate β-adrenergic responsivity, and that the loss of SNO in asthmatic animals contributes to airway tachyphylaxis.

It is generally believed that airway inflammation is central to the pathogenesis of allergic asthma (*1*, *2*). C57BL/6 mice exhibit robust inflammatory responses (fig. S1), which rank highly compared with those of other strains (*25*). It is therefore noteworthy that the total number and composition of leukocytes, including eosinophils, were indistinguishable in ALF from GSNOR$^{-/-}$ and WT mice after PBS or OVA treatment (Fig. 4A) (*21*). Furthermore, interleukin-13 (IL-13) (Fig. 4B) and total serum immunoglobulin E (IgE) (Fig. 4C) were closely comparable in GSNOR$^{-/-}$ and WT mice at baseline and increased equivalently after OVA challenge. In addition, the degree of mucus metaplasia, as determined by periodic acid-Schiff staining (*21*) and mucin gene (*MUC5AC*) expression (Fig. 4D) were comparable in OVA-treated GSNOR$^{-/-}$ and WT mice. Thus, the protection from asthma in *GSNOR*$^{-/-}$ mice does not reflect a suppressed response to

allergen and importantly reveals that SNOs can preserve airway patency in the face of inflammation.

Our findings indicate that the enzyme GSNOR is critical for regulation of airway tone under basal conditions and in response to allergic challenge, and that dysregulation of SNO homeostasis may contribute fundamentally to the asthmatic phenotype. Thus far, the role of NO (and SNO) in asthma has remained a matter of debate. Airway NO is increased (*5*, *6*), but SNOs may be decreased (*18*, *19*) and it has been unclear if NOS activity counters or aggravates airway constriction. We have shown that salutary NO bioactivity in the airways is conveyed largely by SNOs, and in particular, that GSNO protects against AHR. Thus, our results suggest that increases in NO are in fact beneficial when they are channeled adequately into SNOs, and that the key determinant of whether NOS activity exacerbates or protects against asthma may reflect the extent to which SNO-based signaling is preserved (fig. S2). Accordingly, although NOS inhibition is a considered strategy to ameliorate AHR [note trend toward lower AHR in Fig. 2D, consistent with (*26*)], this approach may deleteriously deplete SNOs and thereby exacerbate asthma (Fig. 2, E and F). Our results further suggest that an explanation for the SNO deficit in asthma (*18*, *19*) may be an increase in SNO turnover resulting from increased GSNOR activity and may thus provide a basis for novel therapeutic strategies to alleviate airway obstruction.

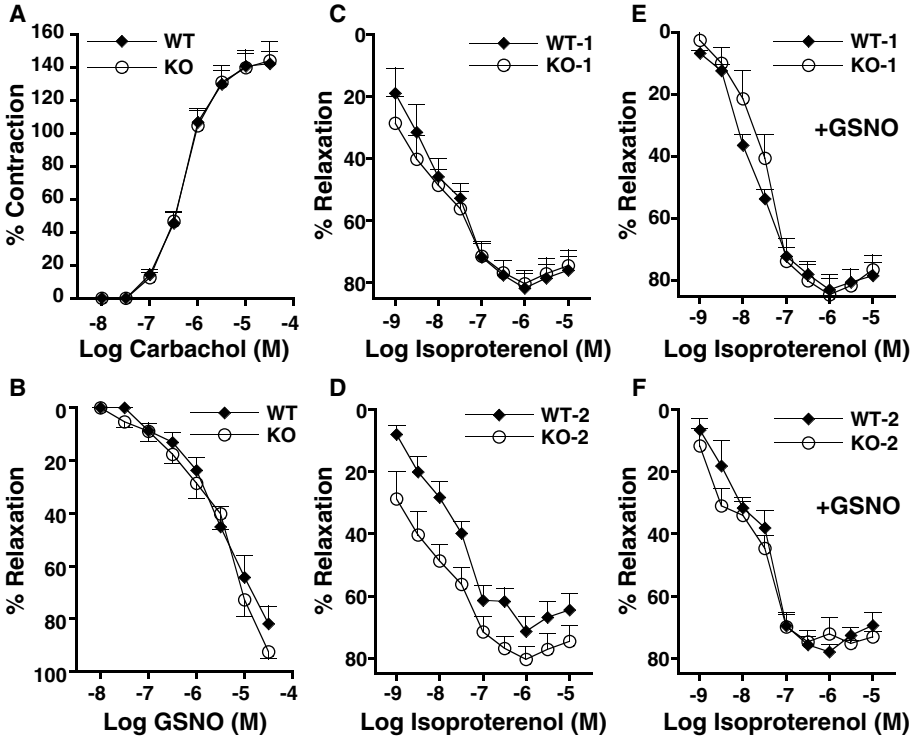

Fig. 3. In vitro contractile and relaxant responses of tracheal rings from WT and GSNOR$^{-/-}$ (KO) mice. Rings were contracted by carbachol (**A**) and relaxed by GSNO (**B**) or isoproterenol (**C** to **F**). Data points are means + SE for $n = 6$ to 8 in all experiments. In (B) to (F), tracheal rings were precontracted with the EC_{50} concentration of carbachol [the concentration in (A) producing 50% of maximal contraction]. Relaxation of tracheal rings in response to the first (WT1 and KO1) (**C**) and second (WT2 and KO2) (D) exposures to isoproterenol. With repeated exposure to isoproterenol, WT rings (WT2) demonstrated a decremental response to β_2-stimulation ($P < 0.03$, WT1 versus WT2; ANOVA), whereas GSNOR$^{-/-}$ rings (KO2) retained their responsivity (P = not significant, KO1 versus KO2). (E and F) Tracheal rings were incubated with 1 μM GSNO for 30 min before exposure to isoproterenol. GSNO-supplemented WT rings show a phenotype indistinguishable from KO (i.e., WT rings no longer desensitize) [(F) versus (D)].

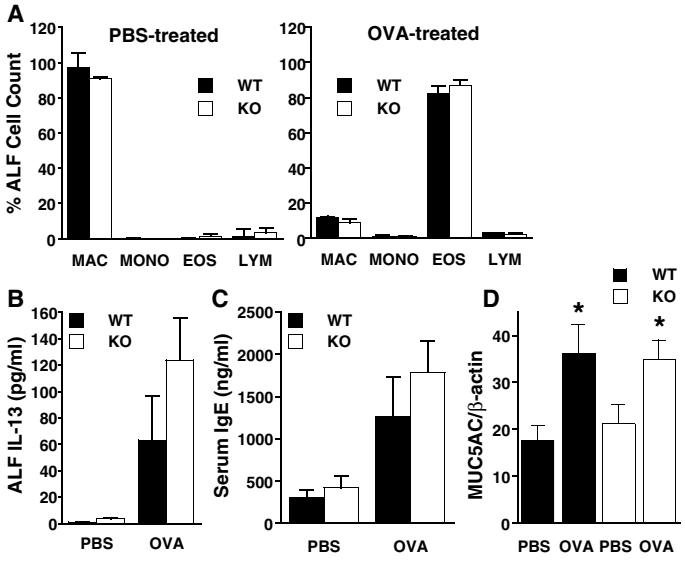

Fig. 4. The inflammatory response to asthmatic challenge was not reduced in GSNOR$^{-/-}$ mice. (**A**) Leukocyte cell differentials (MAC, macrophage; MONO, monocyte; EOS eosinophil; LYM, lymphocyte), (**B**) IL-13 levels in ALF, and (**C**) total serum IgE level were determined for WT (filled) and GSNOR$^{-/-}$ (open) mice that had undergone either control (nonallergic; PBS) or allergic (OVA) exposure. Data are means + SE for $n = 12$ per group. (**D**) *MUC5AC* mRNA levels in whole lung (expressed as a ratio of β-actin mRNA levels) were determined for control (nonallergic; PBS) and allergic (OVA) WT and GSNOR$^{-/-}$ (KO) mice. *MUC5AC* mRNA expression increases comparably in both WT and GSNOR$^{-/-}$ mice after OVA challenge. Values are means + SE, $n = 6$ in each group. *, $P < 0.05$, WT PBS versus WT OVA and KO PBS versus KO OVA.

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27. This paper has been reviewed and approved for release

by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. EPA, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. J.S.S. is a paid consultant to Nitrox LLC, a biotechnology company developing NO-based drugs for disorders of heart, lung, and blood. This work was supported by NIH ES012496 (J.S.S.) and HL004171 (L.G.Q.), and by an award to J.S.S. from the Sandler Program for Asthma Research. We thank N. Coates and W. M. Foster for their assistance.

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Figs. S1 and S2
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Trophic Cascades in a Formerly Cod-Dominated Ecosystem

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Removal of top predators from ecosystems can result in cascading effects through the trophic levels below, completely restructuring the food web. Cascades have been observed in small-scale or simple food webs, but not in large, complex, open-ocean ecosystems. Using data spanning many decades from a once cod-dominated northwest Atlantic ecosystem, we demonstrate a trophic cascade in a large marine ecosystem. Several cod stocks in other geographic areas have also collapsed without recovery, suggesting the existence of trophic cascades in these systems.

Trophic cascades, defined by (i) top-down control of community structure by predators and (ii) conspicuous indirect effects two or more links distant from the primary one, have been intensively researched and controversial for decades (*1*, *2*). The existence of top-down control of ecosystem structure (implied by trophic cascades) creates opportunities for the understanding and manipulation/management of exploited ecosystems, because exploitation is generally focused on top predators (*3*, *4*). From a theoretical perspective, the spatiotemporal balance between the "top-down" (predator dominated) and "bottom-up" (nutrient driven) regulation of ecosystems provides a foundation for understanding their structure, function, and evolution (*5*).

Most ecosystems for which trophic cascades have been shown feature one or more of the following: low species diversity, simple food webs, and small geographic size (*6*, *7*); examples from more complex ecosystems exist (*8*, *9*). This restricted subset of ecological types characterizes many freshwater ecosystems, which constitute most aquatic-based examples of trophic cascades (*6*). Marine continental shelf ecosystems, which generally have large spatial scales, high species diversity, and food web complexity, have not yet revealed unequivocal evidence of trophic cascades. Steele and Collie (*10*) reasoned that continental shelf ecosystems, with their massive changes in predatory fish populations because of exploitation, should provide the most definitive tests of the trophic cascade hypothesis, yet none were found. Reid *et al*. (*5*) found no evidence of trophic cascades in the heavily exploited North Sea, nor did Micheli's (*11*) meta-analysis of 20 open marine systems. However, Worm and Myers's (*12*) meta-analysis of nine continental shelf ecosystems revealed large increases in macroinvertebrate populations following declines in cod (*Gadus morhua*) stocks. Although their findings do not provide evidence of a trophic cascade, they suggest the potential for predation-induced top-down effects in large marine ecosystems. Estes *et al*. (*9*) found evidence of a four-level cascade consisting of killer whales, sea otters, sea urchins, and kelp. However, their time series were limited and the changes in killer whale abundance were unknown. Thus, the evidence for trophic cascades in open ocean systems is equivocal.

Here we provide evidence of a trophic cascade in the large eastern Scotian Shelf ecosystem off Nova Scotia, Canada (Fig. 1) (*13*). The cascade involved four trophic levels and nutrients and was driven by changes in the abundance of large predators (primarily cod) of fish and macroinvertebrates, thereby meeting the requirements of top-down control and indirect effects with multiple links (*1*). Moreover, the cascading effects involved the entire community, rather than only a subset of the species that occupy each of the affected trophic levels.

Consistent with the first criterion of trophic cascades, the system changes were driven by the collapse of the benthic fish community (Fig. 1A). In addition to cod, several other commercially exploited species declined, including haddock (*Melanogrammus aeglefinus*), white hake (*Urophycis tenuis*), silver hake (*Merluccius bilinearis*), pollock (*Pollachius virens*), cusk (*Brosme brosme*), redfish (*Sebastes* sp.), American plaice (*Hippoglossoides platessoides*), yellowtail flounder (*Limanda ferruginea*), thorny skate (*Raja radiata*), and winter skate (*Raja ocellata*). The transition occurred during the mid-1980s and early 1990s and resulted in the virtual elimination of the ecosystem-structuring role of the large-bodied predators that had dominated for centuries (*14*). The abundance of small pelagic fishes and benthic macroinvertebrates [predominantly northern snow crab (*Chionoecetes opilio*) and northern shrimp (*Pandalus borealis*)], once among the primary prey of the benthic fish community (supporting text), increased markedly following the benthic fish collapse (Fig. 1B). The correlations between the benthic fish biomass and small pelagic fishes ($r = -0.61$, $n = 33$ years), snow crab ($r = -0.70$, $n = 24$), and shrimp abundances ($r = -0.76$, $n = 24$) were negative.

Consistent with the second criterion of trophic cascades, there were conspicuous indirect effects resulting from removal of the top predator. As predicted, the correlation between the time series of the benthic fish community (landings) and large (>2 mm), herbivorous zooplankton was positive ($r = 0.45$, $n = 23$), and that for phytoplankton was negative ($r = -0.72$, $n = 24$). These relationships remained equally strong when survey estimates of groundfish biomass were used in place of landings (*13*).

The herbivorous zooplankton abundance series revealed strong evidence of a transition from high to low abundance of large-bodied species from the 1960s and 1970s to the 1990s and beyond (Fig. 1C). This finding is consistent with the enhanced role of size-selective predation on zooplankton by pelagic fishes and early-life stages of shrimp and crab. The abundance of small-bodied (<2 mm) zooplankton remained similar throughout the study period (Fig. 1C). The phytoplankton record (Fig. 1D) revealed a reciprocal pattern: Abundances were low in the 1960s and 1970s and high in the 1990s and beyond. Plankton data ancillary to the continuous plankton recorder data (*13*) revealed a 45% greater abundance of large zooplankton during the early 1980s relative to the late 1990s (Fig. 1C). In contrast, chlorophyll levels were higher in the 1990s relative to the 1980s, but the differences were slight (Fig. 1D). Finally, nitrate concentrations, a major limiting factor in marine systems, showed the expected reciprocal re-

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sponse to changes in phytoplankton abundance (Fig. 1D).

The predatory impact of the expanding grey seal (*Halichoerus grypus*) population (*13*) on the resident cod stock was minor (Fig. 1A) (*15*). Seals appear to have benefited from the cod collapse, which released their forage base (small pelagic fish and benthic invertebrates) from predation. A strong positive correlation between the abundances of small pelagics and grey seals ($r = 0.70$, $n = 33$) and the ongoing exponential rate of increase in the seal population (*16*) support this claim.

Principal component analysis of the series that we used (Fig. 1) and of other biotic, abiotic, and human variables conducted by Choi *et al*. (*17*) has provided statistical evidence and a concise assessment of the change in ecosystem structure. Principal component 1 explained 33% of the variance; its amplitude was positive and nearly constant from 1970 to the mid-1980s, featured a linear transition from the mid-1980s to negative values in the early 1990s, and remained negative and nearly constant since then. Initially (1970 to 1986), benthic fish, possessing good physiological condition, high growth rates, and supporting a >100 × 10^3 metric tons (kt) commercial fishery, dominated; the current period (post-1990) is a pelagic fish/macroinvertebrate–dominated system characterized by poor benthic fish productivity, a <50-kt benthic fish fishery, and a small (<50 kt), but increasing, macroinvertebrate fishery directed at shrimp and snow crab. Several management measures designed to reverse the trend and restore the system to its earlier state have failed. The actions taken included establishment of a fishing closure on two major offshore banks in 1987 that encompass about 15% of the management unit area (*18*), establishment of a moratorium on directed fishing of the dominant benthic fish species (cod, haddock, and pollock) in 1993, and development of new fisheries designed to divert fishing mortality away from the remaining benthic fish species. In 1995, sentinel surveys, supplementing existing scientific surveys, were instituted to monitor and document the anticipated recovery.

Whether the recent ecosystem changes are reversible is an open question. Other factors, both intrinsic and extrinsic, were associated with the ecosystem changes. For example, the expected inverse and reversible relationship between fishing mortality and cod biomass (*13*) that characterized the 1960 to early 1990 period does not hold after 1993 despite the near-elimination of exploitation (Fig. 2A). Physical environmental changes may have contributed to the restructuring of the food web. During the mid-1980s, the average deep-water temperatures declined by ~1°C. This decline started about 4 years before the collapse of cod and other benthic fishes. Recently, temperatures have been normal or above normal without a corresponding increase in benthic fish abundance (Fig. 2B). Vertical stratification of the water column intensified after the collapse and is therefore unlikely to have been a meaningful driver of the changes observed. Stratification has continued to intensify (Fig. 2B), however, and may be contributing to diminished energy flux to the benthic fish community, as revealed by reduced physiological condition and reproductive output (*19*).

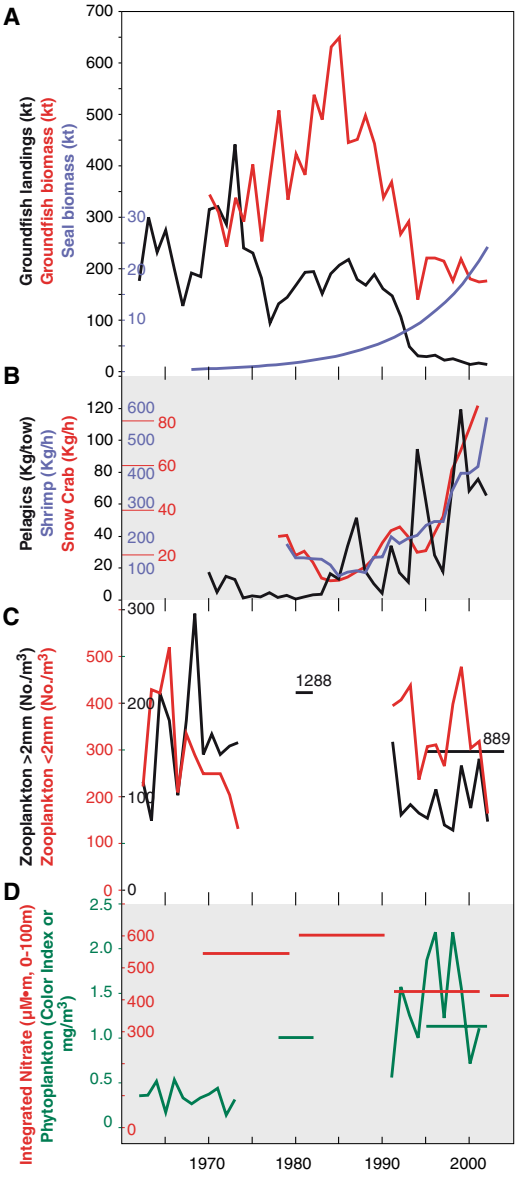

Fig. 1. Illustration of a trophic cascade on the eastern Scotian Shelf across four levels and nutrients. (**A**) Commercial landings of benthic fish species, fishery-independent survey estimates of benthic fish, and population biomass estimates of grey seals. (**B**) The forage base of benthic fish species (and seals), including small pelagic fish species and benthic macroinvertebrates. (**C**) Large (>2 mm) zooplankton, combined abundance of copepodite and adult stages of *Calanus finmarchicus*, *C. glacialis*, and *C. hyperboreous*; small zooplankton, represented by the combined abundance of Calanoid copepods (28 species) other than *Calanus* sp. with body lengths < 2 mm, and large *Calanus* sp. (average number per m^3) from two ancillary sampling programs shown as horizontal lines. (**D**) Phytoplankton color, 0 to 50 m average in situ chlorophyll (mg chlorophyll/m^3), shown as horizontal lines, and 0 to 100 m integrated, dissolved nitrate.

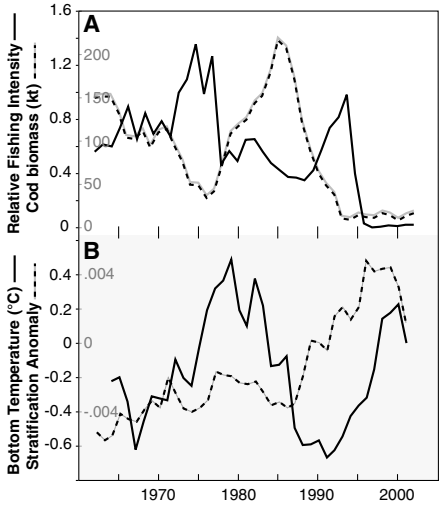

Fig. 2. Intrinsic and extrinsic factors influencing the trophic cascade. (**A**) Time series of cod spawning stock biomass and an index of directed exploitation on the eastern Scotian Shelf. The expected increase in biomass following the reduction of fishing intensity in 1993, as occurred in the late 1970s, is not seen. (**B**) Changes in bottom-water temperatures and vertical stratification [kg/(m^3·m)] of the water column (both shown as 5-year, center-weighted running means) on the eastern Scotian Shelf.

We suspect that this system is not unique. Several cod stocks, inhabiting similar oceanographic regimes (north of 44°N latitude) in the northwest Atlantic where they were the dominant predators, collapsed in the early 1990s (decline by >95% of maximum historical biomass) and failed to respond to complete cessation of fishing [there was one exceptional stock (table S1)]. For example, the current biomass of these stocks has increased only slightly, ranging from 0.4 to 7.0% during the past 10+ years (table S1). Reciprocal relationships between macroinvertebrate biomass and cod abundance in these areas (*12*) suggest that the processes that we document for the Scotian Shelf may have occurred there. On the other hand, the three major cod stocks resident south of 44° N, though reaching historical minimum levels at about the same time as the northerly stocks and experiencing similar intensive fishing pressure, declined by only 50 to 70%; current biomass has increased from 10 to 44% of historical minimum levels. These stocks inhabit different oceanographic regimes with respect to temperature and stratification and do not show the inverse relationship between the biomass of macroinvertebrates and cod found by Worm and Myers (*12*). These geographic differences in cod population dynamics merit additional study.

The changes in top-predator abundance and the cascading effects on lower trophic levels that we report reflect a major perturbation of the eastern Scotian Shelf ecosystem. This perturbation has produced a new fishery regime in which the inflation-adjusted, monetary value of the combined shrimp and crab landings alone now far exceed that of the groundfish fishery it replaced (*13*). From an economic perspective, this may be a more attractive situation. However, one cannot ignore the fundamental importance of biological and functional diversity as a stabilizing force in ecosystems, and indeed in individual populations (*20*), in the face of possible future perturbations (whether natural or human-made). One must acknowledge the ecological risks inherent in "fishing down the food web" (*21*), as is currently occurring on the Scotian Shelf, or the ramifications associated with indirect effects reverberating across levels throughout the food web, such as altered primary production and nutrient cycling.

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Inferences of Competence from Faces Predict Election Outcomes

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We show that inferences of competence based solely on facial appearance predicted the outcomes of U.S. congressional elections better than chance (e.g., 68.8% of the Senate races in 2004) and also were linearly related to the margin of victory. These inferences were specific to competence and occurred within a 1-second exposure to the faces of the candidates. The findings suggest that rapid, unreflective trait inferences can contribute to voting choices, which are widely assumed to be based primarily on rational and deliberative considerations.

Faces are a major source of information about other people. The rapid recognition of familiar individuals and communication cues (such as expressions of emotion) is critical for successful social interaction (*1*). However, people go beyond the inferences afforded by a person's facial appearance to make inferences about personal dispositions (*2*, *3*). Here, we argue that rapid, unreflective trait inferences from faces influence consequential decisions. Specifically, we show that inferences of competence, based solely on the facial appearance of political candidates and with no prior knowledge about the person, predict the outcomes of elections for the U.S. Congress.

In each election cycle, millions of dollars are spent on campaigns to disseminate information about candidates for the U.S. House of Representatives and Senate and to convince citizens to vote for these candidates. Is it possible that quick, unreflective judgments based solely on facial appearance can predict the outcomes of these elections? There are many reasons why inferences from facial appearance should not play an important role in voting decisions. From a rational perspective, information about the candidates should override any fleeting initial impressions. From an ideological perspective, party affiliation should sway such impressions. Party affiliation is one of the most important predictors of voting decisions in congressional elections (*4*). From a voter's subjective perspective, voting decisions are justified not in terms of the candidate's looks but in terms of the candidate's position on issues important to the voter.

Yet, from a psychological perspective, rapid automatic inferences from the facial appearance of political candidates can influence processing of subsequent information about these candidates. Recent models of social cognition and decision-making (*5*, *6*) posit a qualitative distinction between fast, unreflective, effortless "system 1" processes and slow, deliberate, effortful "system 2" processes. Many inferences about other people, including inferences from facial appearance,

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can be characterized as system 1 processes (*7*, *8*). The implications of the dual-process perspective are that person impressions can be formed "on-line" in the very first encounter with the person and can have subtle and often subjectively unrecognized effects on subsequent deliberate judgments.

Competence emerges as one of the most important trait attributes on which people evaluate politicians (*9*–*11*). If voters evaluate political candidates on competence, inferences of competence from facial appearance could influence their voting decisions. To test this hypothesis, we asked naïve participants to evaluate candidates for the U.S. Senate (2000, 2002, and 2004) and House (2002 and 2004) on competence (*12*). In all studies, participants were presented with pairs of black-and-white head-shot photographs of the winners and the runners-up (Fig. 1A) from the election races. If participants recognized any of the faces in a race pair, the data for this pair were not used in subsequent analyses. Thus, all findings are based on judgments derived from facial appearance in the absence of prior knowledge about the person.

As shown in Table 1, the candidate who was perceived as more competent won in 71.6% of the Senate races and in 66.8% of the House races (*13*). Although the data for the 2004 elections were collected before the actual elections (*14*), there were no differences between the accuracy of the prospective predictions for these elections and the accuracy of the retrospective predictions for the 2000 and 2002 elections (*15*). Inferences of competence not only predicted the winner but also were linearly related to the margin of victory. To model the relation between inferred competence and actual votes, we computed for each race the difference in the proportion of votes (*16*). As shown in Fig. 1B, competence judgments were positively correlated with the differences in votes between the candidates for Senate [$r(95) = 0.44$, $P < 0.001$] (*17*, *18*). Similarly, the correlation was 0.37 ($P < 0.001$) for the 2002 House races and 0.44 ($P < 0.001$) for the 2004 races. Across 2002 and 2004, the correlation was 0.40 ($P < 0.001$).

In the previous studies, there were no time constraints on the participants' judgments. However, system 1 processes are fast and efficient. Thus, minimal time exposure to the faces should be sufficient for participants to make inferences of competence. We conducted an experiment in which 40 participants (*19*) were exposed to the faces of the candidates for 1 s (per pair of faces) and were then asked to make a competence judgment. The average response time for the judgment was about 1 s (mean = 1051.60 ms, SD = 135.59). These rapid judgments based on minimal time exposure to faces predicted 67.6% of the actual Senate races ($P < 0.004$) (*20*). The correlation between competence judgments and differences in votes was 0.46 ($P < 0.001$).

The findings show that 1-s judgments of competence suffice to predict the outcomes of actual elections, but perhaps people are making global inferences of likability rather than specific inferences of competence. To address this alternative hypothesis, we asked participants to make judgments on seven different trait dimensions: competence, intelligence, leadership, honesty, trustworthiness, charisma, and likability (*21*). From a simple halo-effect perspective (*22*), participants should evaluate the candidates in the same manner across traits. However, the trait judgments were highly differentiated. Factor analysis showed that the judgments clustered in three distinctive factors: competence (competence, intelligence, leadership), trust (honesty, trustworthiness), and likability (charisma, likability), each accounting for more than 30% of the variance in the data (table S1). More important, only the judgments forming the competence factor predicted the outcomes of the elections. The correlation between the mean score across the three judgments (competence, intelligence, leadership) and differences in votes was 0.58 ($P < 0.001$). In contrast to competence-related inferences, neither the trust-related inferences ($r = -0.09$, $P = 0.65$) nor the likability-related inferences ($r = -0.17$, $P = 0.38$) predicted differences in votes. The correlation between the competence judgment

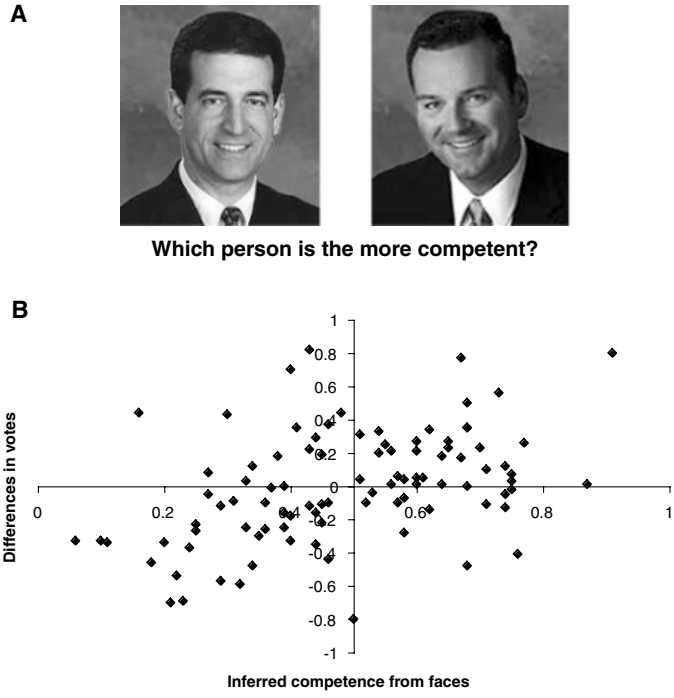

Fig. 1. (**A**) An example of a pair of faces used in the experiments: the 2004 U.S. Senate race in Wisconsin. In all experiments, the positions of the faces were counterbalanced. (**B**) Scatterplot of differences in proportions of votes between the winner and the runner-up in races for the Senate as a function of inferred competence from facial appearance. The upper right and lower left quadrants indicate the correctly predicted races. Each point represents a Senate race from 2000, 2002, or 2004. The competence score on the *x* axis ranges from 0 to 1 and represents the proportion of participants judging the candidate on the right to be more competent than the one on the left. The midpoint score of 0.50 indicates that the candidates were judged as equally competent. The difference in votes on the *y* axis ranges from –1 to +1 [(votes of candidate on the right – votes of candidate on the left)/(sum of votes)]. Scores below 0 indicate that the candidate on the left won the election; scores above 0 indicate that the candidate on the right won the election. [Photos in (A): Capitol Advantage]

Table 1. Percentage of correctly predicted races for the U.S. Senate and House of Representatives as a function of the perceived competence of the candidates. The percentages indicate the races in which the candidate who was perceived as more competent won the race. The χ^2 statistic tests the proportion of correctly predicted races against the chance level of 50%.

Election	Correctly predicted	χ^2
	U.S. Senate	
2000 ($n = 30$)	73.3%	6.53 ($P < 0.011$)
2002 ($n = 33$)	72.7%	6.82 ($P < 0.009$)
2004 ($n = 32$)	68.8%	4.50 ($P < 0.034$)
Total ($n = 95$)	71.6%	17.70 ($P < 0.001$)
	U.S. House of Representatives	
2002 ($n = 321$)	66.0%	33.05 ($P < 0.001$)
2004 ($n = 279$)	67.7%	35.13 ($P < 0.001$)
Total ($n = 600$)	66.8%	68.01 ($P < 0.001$)

alone and differences in votes was 0.55 ($P < 0.002$), and this judgment correctly predicted 70% of the Senate races ($P < 0.028$). These findings show that people make highly differentiated trait inferences from facial appearance and that these inferences have selective effects on decisions.

We also ruled out the possibility that the age, attractiveness, and/or familiarity with the faces of the candidates could account for the relation between inferences of competence and election outcomes. For example, older candidates can be judged as more competent (*23*) and be more likely to win. Similarly, more attractive candidates can be judged more favorably and be more likely to win (*24*). In the case of face familiarity, though unrecognized by our participants, incumbents might be more familiar than challengers, and participants might have misattributed this familiarity to competence (*25*). However, a regression analysis controlling for all judgments showed that the only significant predictor of differences in votes was competence (Table 2). Competence alone accounted for 30.2% of the variance for the analyses of all Senate races and 45.0% of the variance for the races in which candidates were of the same sex and ethnicity. Thus, all other judgments combined contributed only 4.7% of the variance in the former analysis and less than 1.0% in the latter analysis.

Actual voting decisions are certainly based on multiple sources of information other than inferences from facial appearance. Voters can use this additional information to modify initial impressions of political candidates. However, from a dual-system perspective, correction of intuitive system 1 judgments is a prerogative of system 2 processes that are attention-dependent and are often anchored on intuitive system 1 judgments. Thus, correction of initial impressions may be insufficient (*26*). In the case of voting decisions, these decisions can be anchored on initial inferences of competence from facial appearance. From this perspective, in the absence of any other information, voting preferences should be closely related to such inferences. In real-life voting decisions, additional information may weaken the relation between inferences from faces and decisions but may not change the nature of the relation.

To test this hypothesis, we conducted simulated voting studies in which participants were asked to choose the person they would have voted for in a political election (*27*). If voting preferences based on facial appearance derive from inferences of competence, the revealed preferences should be highly correlated with competence judgments. As shown in Fig. 2, the correlation was 0.83 ($P < 0.001$) (*28*). By comparison, the correlation between competence judgments and actual differences in votes was 0.56 ($P < 0.001$). These findings suggest that the additional information that voters had about the candidates diluted the effect of initial impressions on voting decisions. The simulated votes were also correlated with the actual votes [$r(63) = 0.46$, $P < 0.001$] (*29*, *30*). However, when controlling for inferences of competence, this correlation dropped to 0.01 ($P = 0.95$), which suggests that both simulated and actual voting preferences were anchored on inferences of competence from facial appearance.

Our findings have challenging implications for the rationality of voting preferences, adding to other findings that consequential decisions can be more "shallow" than we would like to believe (*31*, *32*). Of course, if trait inferences from facial appearance are correlated with the underlying traits, the effects of facial appearance on voting decisions can be normatively justified. This is certainly an empirical question that needs to be addressed. Although research has shown that inferences from thin slices of nonverbal behaviors can be surprisingly accurate (*33*), there is no good evidence that trait inferences from facial appearance are accurate (*34–39*). As Darwin recollected in his autobiography (*40*), he was almost denied the chance to take the historic Beagle voyage—the one that enabled the main observations of his theory of evolution—on account of his nose. Apparently, the captain did not believe that a person with such a nose would "possess sufficient energy and determination."

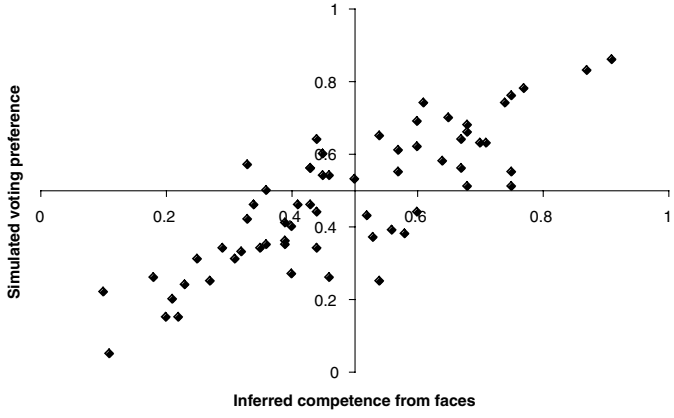

Fig. 2. Scatterplot of simulated voting preferences as a function of inferred competence from facial appearance. Each point represents a U.S. Senate race from 2000 or 2002. One group of participants was asked to cast hypothetical votes and another group was asked to judge the competence of candidates. Both the competence score and the voting preference score range from 0 to 1. The competence score represents the proportion of participants judging the candidate on the right to be more competent than the one on the left. The preference score represents the proportion of participants choosing the candidate on the right over the one on the left. The midpoint score of 0.50 on the *x* axis indicates that the candidates were judged as equally competent. The midpoint score of 0.50 on the *y* axis indicates lack of preference for either of the candidates.

Table 2. Standardized regression coefficients of competence, age, attractiveness, and face familiarity judgments as predictors of differences in proportions of votes between the winner and the runner-up in races for the U.S. Senate in 2000 and 2002. Matched races are those in which both candidates were of the same sex and ethnicity.

Predictor	Differences in votes between winner and runner-up	
	All races	Matched races
Competence judgments	0.49 ($P < 0.002$)	0.58 ($P < 0.002$)
Age judgments	0.26 ($P < 0.061$)	0.07 ($P = 0.62$)
Attractiveness judgments	0.07 ($P = 0.63$)	0.08 ($P = 0.62$)
Face familiarity judgments	–0.05 ($P = 0.76$)	0.03 ($P = 0.86$)
Accounted variance (R^2)	34.9%	45.8%
Number of races	63	47

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10. In one of our studies, 143 participants were asked to rate the importance of 13 different traits in considering a person for public office. These traits included competence, trustworthiness, likability, and 10 additional traits mapping into five trait dimensions that are generally believed by personality psychologists to explain the structure of personality: extraversion, neuroticism, conscientiousness, agreeableness, and openness to experience (*11*). Competence was rated as the most important trait. The mean importance assigned to competence was 6.65 (SD = 0.69) on a scale

ranging from 1 (not at all important) to 7 (extremely important). The importance assigned to competence was significantly higher than the importance assigned to any of the other 12 traits (Ps < 0.005).
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13. For the House races in 2002, we were able to obtain pictures of both the winner and the runner-up for 321 of the 435 races. For the House races in 2004, we were able to obtain pictures for 279 of the 435 races (*12*).
14. In the studies involving these races, we used photographs of the Democratic and Republican candidates (*12*).
15. In addition, the accuracy of the predictions was not affected by the race and sex of the candidates. This is important because participants might have used race and sex stereotypes to make competence judgments for contests in which the candidates were of different sexes and races. For example, in such contests Caucasian male candidates were more likely to win. However, if anything, competence judgments predicted the outcomes of elections in which the candidates were of the same sex and race (73.1% for the Senate and 68.5% for the House) more accurately than elections in which they were of different sexes and races (67.9% and 64.3%, respectively). This difference possibly reflects participants' social desirability concerns when judging people of different race and sex.
16. For races with more than two candidates, we standardized this difference so that it was comparable to the difference in races with two candidates. Specifically, the difference between the votes of the winner and those of the runner-up was divided by the sum of their votes.
17. From the scatterplot showing the relation between competence judgments and votes for Senate (Fig. 1B), seven races (three in the lower right quadrant and four in the upper left quadrant) could be identified as deviating from the linear trend. It is a well-known fact that incumbents have an advantage in U.S. elections (*18*). In six of the seven races, the incumbent won but was judged as less competent. In the seventh race (Illinois, 2004) there was no incumbent, but the person who won, Barack Obama, was the favorite long before the election. Excluding these seven races, the correlation between competence judgments and differences in votes increased to 0.64 ($P < 0.001$). Although incumbent status seemed to affect the strength of the linear relation between inferences of competence and the margin of victory, it did not affect the prediction of the outcome. Competence judgments predicted the outcome in 72.9% of the races in which the incumbent won, in 66.7% of the races in which the incumbent lost, and in 68.8% of the cases in which there was no incumbent ($\chi^2 < 1.0$ for the difference between these percentages; $P = 0.89$).
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19. A bootstrapping data simulation showed that increasing the sample size to more than 40 participants does not improve the accuracy of prediction substantially (*12*) (fig. S1).
20. Given the time constraints in this study, to avoid judgments based on salient differences such as race and sex, we used only Senate races (2000, 2002, and 2004) in which the candidates were of the same sex and race.
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27. For these studies, we used the 2000 and 2002 Senate races (*12*).
28. An additional analysis from a study in which participants made judgments of the candidates for the Senate (2000 and 2002) on 13 different traits [see (*10*) for the list of traits] provided additional evidence that inferences of competence were the key determinants of voting preferences in this situation. We regressed voting preferences on the 13 trait judgments. The only significant predictor of these preferences was the judgment of competence [$\beta = 0.67$, $t(49) = 4.46$, $P < 0.001$].
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34. There is some evidence that judgments of intelligence from facial appearance correlate modestly with IQ scores (*35*). However, these correlations tend to be small [e.g., <0.18 in (*35*)], they seem to be limited to judgments of people from specific age groups (e.g., puberty), and the correlation is accounted for by the judges' reliance on physical attractiveness. That is, attractive people are perceived as more intelligent, and physical attractiveness is modestly correlated with IQ scores.
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Materials and Methods
SOM Text
Fig. S1
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TLR11 Activation of Dendritic Cells by a Protozoan Profilin-Like Protein

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Mammalian Toll-like receptors (TLRs) play an important role in the innate recognition of pathogens by dendritic cells (DCs). Although TLRs are clearly involved in the detection of bacteria and viruses, relatively little is known about their function in the innate response to eukaryotic microorganisms. Here we identify a profilin-like molecule from the protozoan parasite *Toxoplasma gondii* that generates a potent interleukin-12 (IL-12) response in murine DCs that is dependent on myeloid differentiation factor 88. *T. gondii* profilin activates DCs through TLR11 and is the first chemically defined ligand for this TLR. Moreover, TLR11 is required in vivo for parasite-induced IL-12 production and optimal resistance to infection, thereby establishing a role for the receptor in host recognition of protozoan pathogens.

Mammalian Toll-like receptors (TLRs) play a fundamental role in the initiation of immune responses to infectious agents through their recognition of conserved microbial molecular patterns (*1*). TLR signaling in antigen-presenting cells, such as dendritic cells (DCs), results in the production of cytokines and costimulatory molecules that are required for initiation of the adaptive immune response (*2*, *3*). Human and mouse TLR family members have been shown to have distinct ligand specificities, recognizing molecular structures such as lipopeptide (TLR2) (*4*), lipopolysaccharide (TLR4) (*5*, *6*), flagellin (TLR5) (*7*), double- and single-stranded RNA (TLR3 and TLR7) (*8*–*11*), and CpG motifs of DNA (TLR9) (*12*). Although several TLRs have been shown to be important for immune responses to microbial products in vitro, their role in host resistance to infection appears to be complex and not

readily attributed to the function of a single TLR (*13*). Of particular help in assessing the role of TLR functions has been a mouse carrying a deletion in the gene encoding myeloid differentiation factor 88 (MyD88), an adaptor molecule that is essential for most TLR, interleukin-1 (IL-1), and IL-18 signaling (*14*). MyD88$^{-/-}$ mice have been shown to be acutely susceptible to a wide variety of bacterial, fungal, protozoan, and viral agents (*13*).

A critical host mediator produced in response to TLR activation is IL-12. This cytokine is synthesized by DCs, macrophages, and neutrophils and plays a pivotal role in the production of interferon-γ (IFN-γ), which in turn activates antimicrobial effector cells (*15*). In previous studies, we have shown that IL-12 is essential for host resistance to the protozoan parasite *Toxoplasma gondii* and that DCs produce large quantities of the cytokine in response to stimulation with this pathogen (*16*, *17*). Both host resistance to *T. gondii* and parasite-induced IL-12 production by DCs have been shown to require MyD88, which strongly suggests the involvement of TLR signaling (*18*). Nevertheless, the question of which TLR molecule or molecules govern *T. gondii*–induced IL-12 production by DCs remained unanswered.

In addition to MyD88 signaling, activation of DCs by *T. gondii* has been shown to involve ligation of the chemokine receptor CCR5 by a *T. gondii* protein, cyclophilin-18 (C-18) (*19*, *20*). Because stimulation by C-18 does not explain the MyD88 dependence of the IL-12 response to the parasite, we searched for an additional ligand in *T. gondii* that might trigger DC IL-12 production by a MyD88-dependent but CCR5-independent pathway.

We used DCs from CCR5-deficient mice as responder cells in purifying an IL-12–inducing fraction from STAg, a soluble extract of the tachyzoite stage of the parasite (*17*). Pilot studies indicated that the cytokine-stimulating activity was protease-sensitive (fig. S1A), and we therefore fractionated STAg by gel filtration (Fig. 1A). A low-molecular-weight peak consisting of the two most active fractions (B7 and B8) was further separated, yielding a single fraction (fig. S1B) that stimulated high levels of IL-12 production and contained a single silver-stained band on SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1B), which was analyzed by mass spectrometry, followed by Sequest peptide mapping (fig. S1C). A high-scoring match was found for a tryptic peptide in the *T. gondii* clustered expressed sequence tag (EST) database (http://ToxoDB.org). Based on its complete sequence, the *T. gondii* protein identified by us has a predicted molecular mass of 17.5 kD and contains consensus motifs shared by profilins, a class of actin-binding proteins (*21*). Database searches performed with the *T. gondii* profilin (PFTG) sequence revealed significant homology only with profilin genes that are present in other apicomplexan protozoa (Fig. 1C and figs. S2 and S3).

The cloned *T. gondii* profilin-like gene was used to transform *Escherichia coli*, and

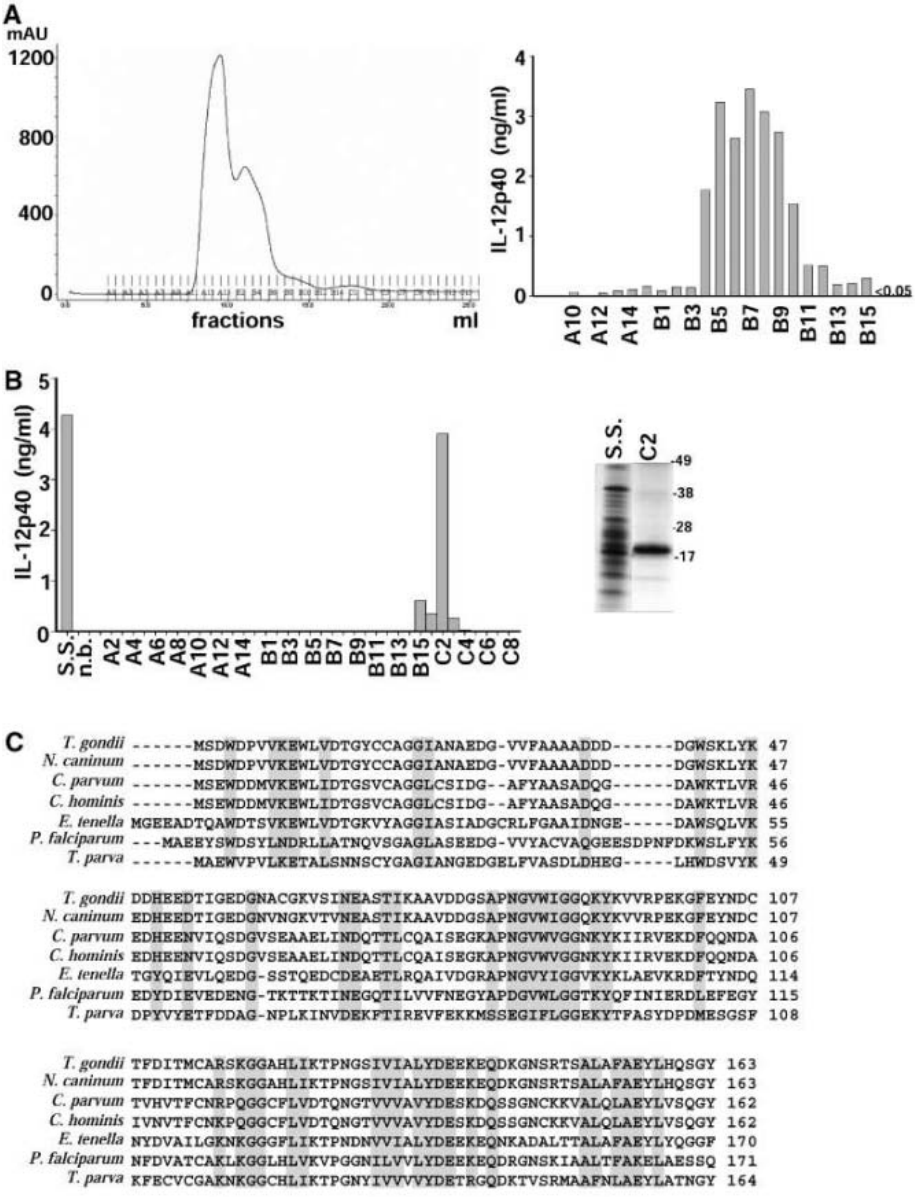

Fig. 1. Isolation of a major IL-12–inducing protein from *T. gondii*. (**A**) Initial separation of soluble tachyzoite extract (STAg) on Superdex-75 Sepharose and assay of individual fractions for their ability to stimulate IL-12 production by splenic DCs from CCR5$^{-/-}$ mice. mAU, relative milliabsorbance at 280 nm. (**B**) Further purification by Mono Q anion-exchange chromatography of fractions B7 and B8 from the first separation. The inset at right shows a silver-stained SDS-PAGE analysis of the fraction (C2) with peak IL-12–inducing activity compared with the starting sample (S.S.). (**C**) Amino acid sequence alignment of the cloned *T. gondii* IL-12–inducing protein with its nearest homologs in the National Center for Biotechnology Information database. All these sequences are profilin-like proteins from the related apicomplexan parasites *Neospora caninum* (93% homology), *Cryptosporidium parvum* (63% homology), *Cryptosporidium hominis* (63% homology), *Eimeria tenella* (67% homology), *Plasmodium falciparum* (58% homology), and *Theileria parva* (53% homology).

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the resulting lysate induced IL-12 production by DCs at approximately 20 times the level seen with controls (fig. S4A). The purified PFTG recombinant protein induced potent IL-12 p40 and IL-12 p70 responses from splenic DCs and was approximately 100 times more active in a dose-response analysis than unfractionated STAg (Fig. 2). In contrast, recombinant C-18 was found to be less active than STAg in inducing IL-12 production (*20*, *22*).

T. gondii is known to preferentially induce IL-12 production in CD8α$^+$ DCs (*17*), and a similar DC subset restriction was observed for IL-12 (Fig. 2 and fig. S4B), tumor necrosis factor (TNF), and IL-6 induction by PFTG (fig. S4C). Consistent with the known MyD88 dependence of *T. gondii*–induced cytokine production (*18*), DCs from MyD88$^{-/-}$ mice displayed severely impaired IL-12, TNF, and IL-6 responses to PFTG (Fig. 2 and fig. S4, B and C).

Two distinct TLRs have been implicated in the recognition of protein ligands. TLR5 has been shown to be triggered by bacterial flagellin, whereas TLR11 signaling is stimulated by protease-sensitive molecules in uropathogenic bacteria (*7*, *23*). We observed that TLR11 but not TLR5 transfectants displayed dose-dependent nuclear factor κB (NF-κB) activation when stimulated with PFTG (Fig. 3A). We next compared the response to PFTG and STAg stimulation of splenic DCs from TLR11$^{-/-}$ mice with the response of DCs from other TLR-deficient mice. Unlike DCs from wild-type animals, TLR11$^{-/-}$ DCs failed to produce IL-12p40 (Fig. 3B), TNF, or IL-6 (*22*) in response to either PFTG or STAg at doses as high as 1 μg/ml, whereas no significant defects in cytokine response were observed with comparable DC populations from TLR2- (*4*), TLR3- (*8*), TLR4- (*6*), TLR7- (*24*), or TLR9-deficient (*12*) mice (fig. S5A) (*22*). Fluorescence-activated cell sorter analysis confirmed that the defective response observed with the TLR11$^{-/-}$ cell population was not the result of a deficiency in CD8α$^+$CD11c$^+$ DCs (fig. S5B). TLR11 mRNA was detected in both CD8α$^+$ and CD8α$^-$ DCs, although at higher levels in the former subpopulation (fig. S5C).

To investigate whether the PFTG-TLR11 interaction was also critical for IL-12 induction in vivo, wild-type and TLR11$^{-/-}$ mice were injected with STAg or PFTG and examined for serum cytokine levels. In contrast to the control animals, which produced a vigorous IL-12 response, TLR11$^{-/-}$ mice failed to produce detectable levels of the cytokine (Fig. 4A). Moreover, splenic DCs in STAg- or PFTG-injected TLR11$^{-/-}$ mice as well as MyD88$^{-/-}$ mice failed to migrate into T cell areas (Fig. 4B) or stain with monoclonal antibody to IL-12 (*22*), which is the response typically seen in wild-type animals (*17*). Finally, to test whether TLR11 also governs IL-12–dependent host resistance to live parasite infection, control and TLR11$^{-/-}$ mice were infected with ME-49, an avirulent *T. gondii* strain. In contrast to infected wild-type animals, which produced high levels of circulating IL-12p40 and IFN-γ, *T. gondii*–exposed TLR11$^{-/-}$ mice displayed low serum IL-12 levels, which were only slightly elevated above those seen in MyD88$^{-/-}$ animals, as well as reduced levels of IFN-γ (Fig. 4C). Nevertheless, in contrast to MyD88$^{-/-}$ animals, the infected TLR11$^{-/-}$ mice survived the acute phase of infection. However, the TLR11$^{-/-}$ mice clearly showed impaired resistance, as was made evident by a nearly fivefold elevation in the numbers of brain tissue cysts relative to wild-type animals measured during the chronic phase of infection (Fig. 4D).

The results presented here identify the first chemically defined ligand for TLR11 and demonstrate previously unappreciated roles for TLR11 signaling in pathogen-induced cytokine production by DCs, as well as host resistance to protozoan infection. The initial observation that proteinase K digestion destroys the ability of uropathogenic *E. coli* lysates to stimulate TLR11 had suggested that this receptor recognizes proteins (*23*). Our findings confirm this hypothesis by demonstrating direct TLR11 stimulation by a recombinant parasite protein. Although studies are in progress to determine whether structurally related ligands also exist in uropathogenic bacteria, *T. gondii* profilin homologs are clearly present in other apicomplexan parasites (Fig. 1C and figs. S2 and S3). In this regard, we have found that recombinant profilins from *Cryptosporidium parvum* and *Plasmodium falciparum* induce IL-12 production in varying degrees (fig. S6), and a profilin cloned from *Eimeria tenella* was recently

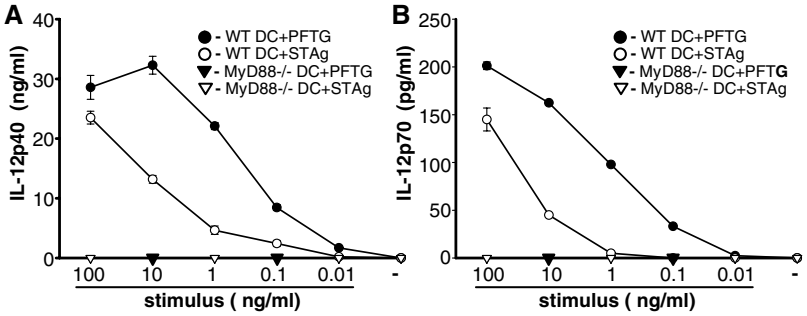

Fig. 2. Dose response of wild-type versus MyD88$^{-/-}$ DCs to PFTG in comparison with the response to unfractionated STAg. Sort-purified CD8α$^+$CD11c$^+$ splenic DCs from either C57BL/6 or MyD88$^{-/-}$ mice were exposed to graded doses of recombinant PFTG or STAg, and IL-12p40 (**A**) and IL-12p70 (**B**) were measured in supernatants after overnight culture. The data shown are the mean ± SD of triplicate assays performed at each dilution and are representative of four experiments performed.

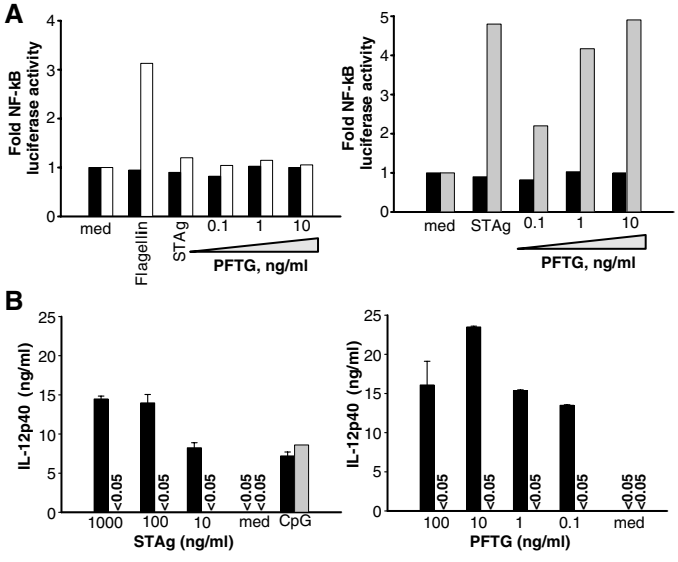

Fig. 3. PFTG is a TLR11 ligand. (**A**) PFTG stimulates TLR11- but not TLR5-transfected cells. CHO-K1 cells transfected with empty vector (black bars), TLR5-expressing vector (white bars, left panel), or TLR11-expressing vector (gray bars, right panel) were stimulated with increasing concentrations of recombinant PFTG, STAg (1 μg/ml), flagellin C (1 μg/ml, InvivoGen), or left untreated in medium (med), and NF-κB luciferase activity was measured 4 hours later. The data shown are means of duplicate points from a representative experiment out of two performed. (**B**) TLR11 is required for the IL-12 response of DCs to both PFTG and STAg. Total splenic DCs from wild-type (black bars) or TLR11$^{-/-}$ (gray bars) mice were exposed to graded doses of either STAg, PFTG, or CpG (10 μM), and IL-12p40 production was measured after overnight incubation. The data shown are the mean ± SD of triplicate assays performed at each dilution and are representative of three experiments performed (<0.05 = below the limit of detection).

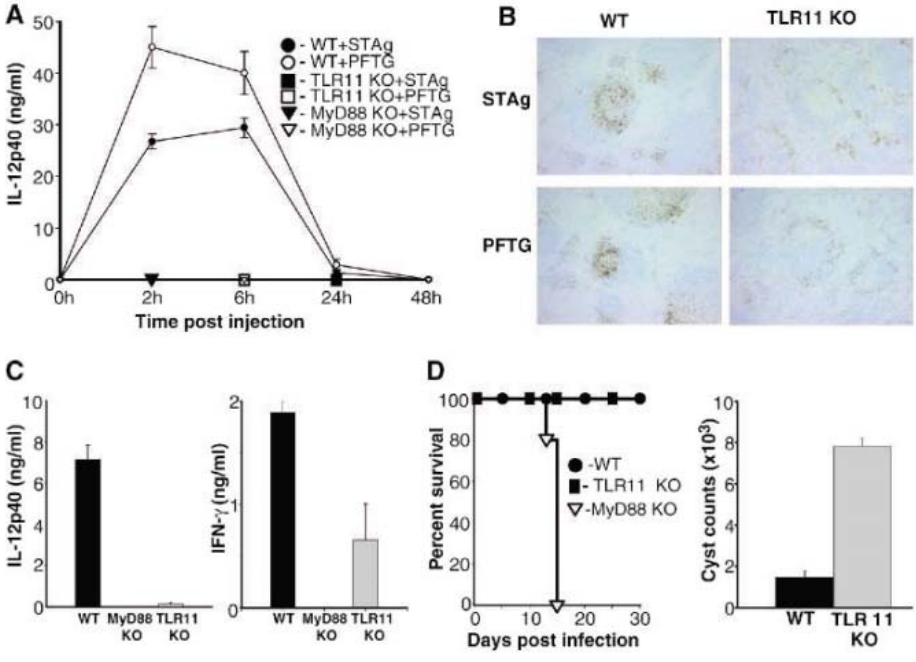

Fig. 4. TLR11 plays a major role in the in vivo IL-12 response to STAg and PFTG as well as to infection with live *T. gondii*. (**A**) TLR11 is required for IL-12 production in response to injected STAg or PFTG. TLR11 knockout (KO), littermate controls, and MyD88 KO mice (n = 3 or 4 per group) were injected intraperitoneally with 10 μg of STAg or recombinant PFTG and were bled at the time points indicated. The data shown are the group means ± SD from duplicate IL-12p40 enzyme-linked immunosorbent assay (ELISA) measurements performed on each mouse. (**B**) TLR11 is required for the migration of splenic DCs induced by STAg or PFTG. TLR11$^{-/-}$ or wild-type (WT) animals were injected with STAg or PFTG as described above, and spleens were removed 6 hours later for immunocytochemistry. The brown stain indicates CD11c^{+} cells, whereas the blue stain marks B220^{+} cells to localize B cell areas in the spleen. The images shown are representative of multiple sections examined in three or four mice per group. Sections from injected MyD88$^{-/-}$ mice were indistinguishable from those from TLR11$^{-/-}$ mice (*22*). (**C**) TLR11$^{-/-}$ mice infected with an avirulent strain of *T. gondii* display deficient IL-12 and IFN-γ production. C57BL/6, MyD88$^{-/-}$, and TLR11$^{-/-}$ mice (five animals per group) were infected with an average of 20 cysts per mouse of the ME49 strain of *T. gondii*, and serum IL-12p40 and IFN-γ responses were measured 5 days later by ELISA. The data shown are pooled from two individual experiments that gave comparable results. (**D**) Cumulative survival of the mice shown in (C). All surviving animals were killed on day 30 and brain cysts counts were determined as a measure of infection level. The cyst numbers shown are the pooled means ± SD from the two experiments performed.

shown to have similar activity (*25*). Together these findings suggest that TLR11 may broadly recognize apicomplexan profilins.

Host resistance to *T. gondii* in mice has previously been shown to depend on both MyD88 and IL-12 production, and DCs are a major source of this cytokine (*16*–*18*). The data presented here establish TLR11 as the major pattern recognition receptor involved in the triggering of DC IL-12 production by *T. gondii* and identify the first parasite-derived protein TLR ligand. Although DC IL-12 production appeared to be almost totally impaired in infected TLR11$^{-/-}$ mice, these animals, unlike either MyD88$^{-/-}$ or IL-12$^{-/-}$ mice (*18*), retained partial resistance to challenge and survived the acute phase of the infection, most likely because of a residual IFN-γ response. This unexpected resistance may reflect the contribution of other MyD88-dependent IL-1/TLR family members (*13*) functioning together with the small amount of IL-12 still produced in the infected TLR11$^{-/-}$ mice. In this regard, it is of interest that TLR2$^{-/-}$ mice show increased susceptibility to *T. gondii* infection, but only when abnormally high challenge doses are used (*26*).

Rodents are important intermediate hosts in the natural life cycle of *T. gondii*, and although it causes disease in humans, the parasite is also a major pathogen of livestock. Although our results establish a role for TLR11 in the response of mice to *T. gondii*, human TLR11 is nonfunctional because of the presence of a stop codon in the gene (*23*). At present, it is not clear whether TLR11 recognition of *T. gondii* is of importance in limiting infection in other mammalian species or whether humans use alternative pattern recognition receptors in the innate response to *T. gondii*.

The *T. gondii* profilin-like molecule described here is the second known microbial protein recognized by a TLR, the first being flagellin, the ligand for TLR5 (*7*). Flagellin is required for bacterial motility, and the region of the molecule involved in TLR5 interaction is highly conserved and necessary for this function (*27*). Profilin-like molecules structurally related to PFTG are present in a number of apicomplexan protozoa. Although their exact cellular functions have not been established, their predicted actin-binding activity suggests that, like flagellin, they may be involved in parasite motility and/or invasion (*28*). Studies are in progress to both define the structural domain in PFTG that is necessary for TLR11 interaction and to determine whether it is phylogenetically conserved among related protozoa and therefore serves as a pathogen-associated molecular pattern for this group of eukaryotes.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S6
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Microsatellite Instability Generates Diversity in Brain and Sociobehavioral Traits

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Repetitive microsatellites mutate at relatively high rates and may contribute to the rapid evolution of species-typical traits. We show that individual alleles of a repetitive polymorphic microsatellite in the 5′ region of the prairie vole *vasopressin 1a receptor* (*avpr1a*) gene modify gene expression in vitro. In vivo, we observe that this regulatory polymorphism predicts both individual differences in receptor distribution patterns and socio-behavioral traits. These data suggest that individual differences in gene expression patterns may be conferred via polymorphic microsatellites in the cis-regulatory regions of genes and may contribute to normal variation in behavioral traits.

Social behavior evolves rapidly as evidenced by the diversity of species-typical social structures among closely related species; however, the underlying mechanisms of this rapid evolution are currently unknown. Evolution of species-typical behavioral traits requires behavioral diversity and a polymorphic genetic mechanism producing such diversity. The high levels of polymorphism in repetitive DNA sequences (microsatellites) make them useful as markers to distinguish among individuals. Expanded repeats in coding regions of genes are responsible for diseases such as Huntington's disease and the spinocerebellar ataxias (*1*). Recently, expansion and contraction in the coding regions of several developmental genes has been hypothesized to underlie the rapid and continuous evolution of snout morphology in domesticated dog breeds (*2*). Although there is not as much empirical evidence, variation in regulatory regions of genes likely plays a critical role in the generation of morphological variation by altering the timing and location of gene expression (*3*, *4*). Microsatellites in the cis-regulatory regions of genes may significantly enhance the rate of evolution of gene expression patterns and selectable phenotypic traits (*5*–*7*).

Rodents of the genus *Microtus* (voles) show dramatic species differences in social structure (*8*). Prairie voles form lifelong attachments with a mate, are biparental, and show high levels of social interest (*9*). In contrast, the closely related montane vole does not pair bond, the males do not contribute to parental care, and they appear socially indifferent (*10*). Species differences in the pattern of vasopressin 1a receptor (V1aR) expression in the brains of these species contribute to the species differences in social structure (*11*–*13*). The species-specific patterns of V1aR expression appear to be regulated by differences in a microsatellite in the 5′ regulatory region of the gene encoding V1aR (*avpr1a*). This microsatellite is highly expanded in prosocial prairie and pine voles, consisting of several repeat blocks interspersed with nonrepetitive sequences (Fig. 1, A and B), compared with a very short version in the asocial montane and meadow voles (*14*). The species differences in this microsatellite have functional consequences in cell culture: In transcription reporter assays, changes in the microsatellite locus modify luciferase reporter activity in a cell-type–dependent manner (*15*).

Because microsatellites offer a mechanism of continuous phenotypic variation, it follows that there should also be a microsatellite-phenotype relation within a species. Accordingly, within the prairie vole species, there is variation in social behavior across different geographical populations and even within the same population in communal pens (*16*, *17*). In addition, the length of the *avpr1a* microsatellite is variable [Fig. 1, B and C; (*15*, *18*)] as are V1aR distribution patterns across individuals within the prairie vole species (*19*), although levels of intraspecific variation are lower than the levels of interspecific variation in both genotype and phenotype (*19*, *20*). Whereas microsatellite length is as-

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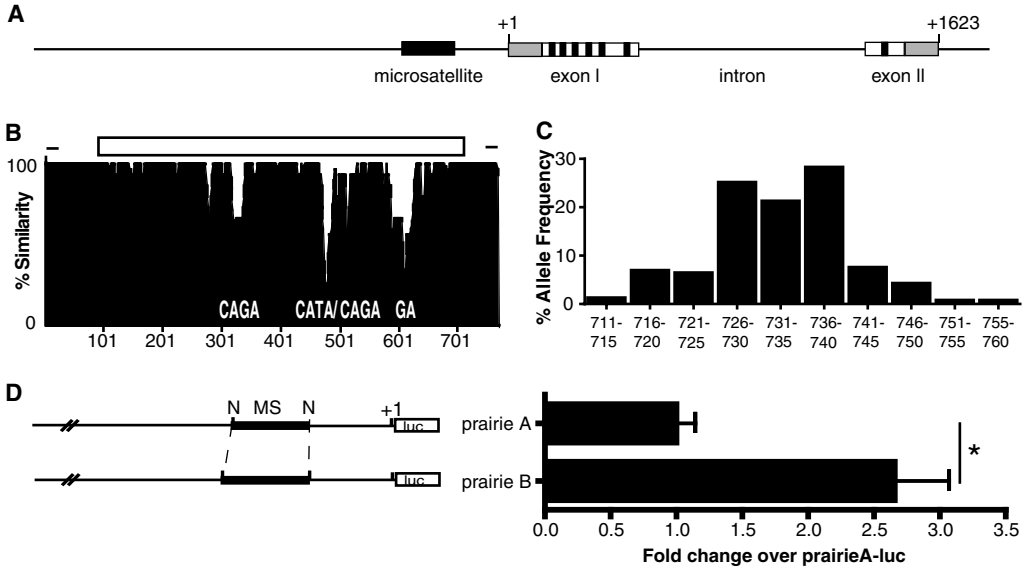


Fig. 1. The prairie vole *avpr1a* 5′ regulatory region contains a functional polymorphic microsatellite. (**A**) The prairie vole microsatellite is located ~500 bp upstream of the *avpr1a* transcription start site. (**B**) The prairie vole-specific microsatellite, indicated by the white box, is ~600 bp long. PCR primers used for genotyping, indicated by dashes, generate PCR products ranging from 710 to 760 bp in length. This alignment of various alleles from 9 prairie voles reveals that the 5′ end is relatively conserved among prairie voles, whereas the 3′ end is repetitive and highly polymorphic. The relative positions of various repeat motifs are indicated on the alignment. Expansion and contraction at these repeat motifs contributes to the length variation in microsatellite alleles. (**C**) A histogram of allele frequencies from our laboratory breeding colony of voles reveals high levels of length polymorphism. (**D**) Two different common alleles of this microsatellite differentially regulate gene expression in rat A7r5 cells. The length genotype of prairie A was 727 bp; the allele length of prairie B was 746 bp. Bars in (D) represent means + SEM (n = 6 per experiment).

sociated with species differences in V1aR expression and social structure, we hypothesize that intraspecific variation in microsatellite length generates intraspecific variation in V1aR expression and, consequently, sociobehavioral traits.

To determine whether or not intraspecific variation in the microsatellite itself is sufficient to change gene expression, we created luciferase reporter constructs containing ~3.5 kb of prairie vole *avpr1a* 5′ regulatory region, including the microsatellite locus. Holding the rest of the 5′ regulatory region constant, we interchanged two different prairie vole alleles of this microsatellite to test them against each other for their ability to drive the luciferase reporter in A7r5 cells. The two alleles only differ in length by 19 base pairs (bp), primarily attributable to an expanded GA repeat in the longer allele. In three independent cell culture experiments, the longer allele had significantly increased levels of luciferase activity compared with the shorter allele, demonstrating that intraspecific variation in the microsatellite itself modifies gene expression (Fig. 1D, two-tailed Student's *t* test with Welch's correction, $P < 0.0001$). Although the long allele showed higher levels of expression in this particular cell line that we tested, other evidence indicates that the effect may be cell-type dependent.

We next created 25 breeding pairs of prairie voles based on the length of their *avpr1a* microsatellite polymorphism to generate testable offspring that were homozygous for either longer or shorter than the average allele length. Two out of three litter cohorts

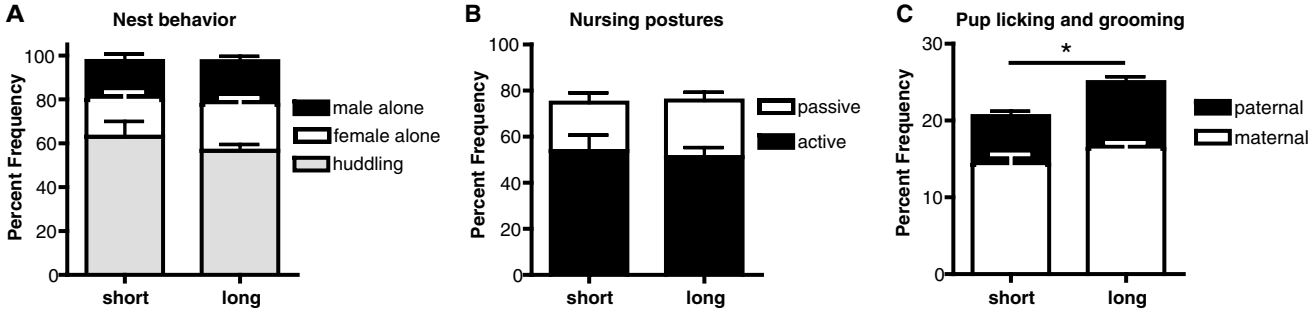
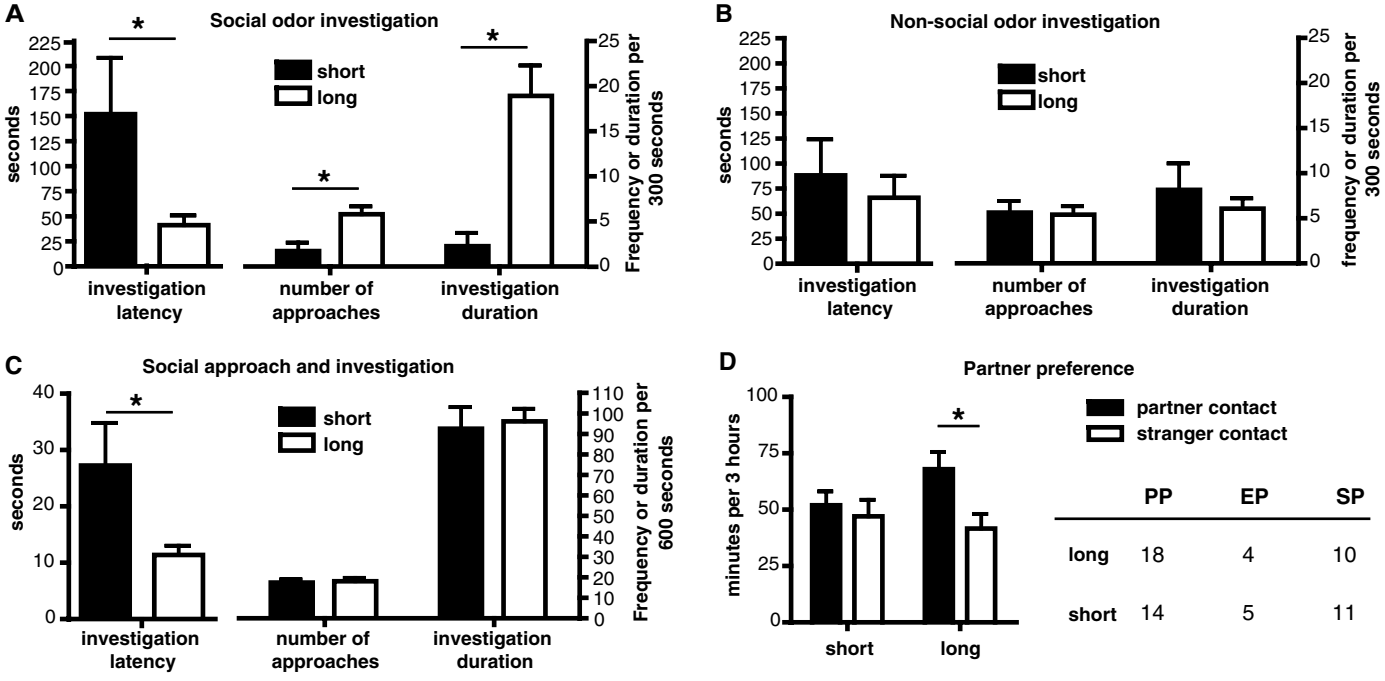


Fig. 2. Genotype differences in male but not female parental care. (**A**) Huddling rates and the frequency of each parent alone on the nest were not different between the two genotype groups. (**B**) Maternal nursing postures were not different between the two genotype groups. (**C**) Total rates of pup licking and grooming were higher in the long-allele group, and this was attributed to differences in grooming by the males, but not the females. Bars in (A, B, and C) represent means + SEM. $n = 6$ short allele pairs, $n = 11$ long allele pairs



	PP	EP	SP
long	18	4	10
short	14	5	11

Fig. 3. Long-allele males have a greater probability of social engagement and bonding behavior. (**A**) Long-allele males ($n = 12$) more quickly approached a novel social odor, approached the social odor more frequently, and spent a longer amount of time investigating the social odor than short-allele males ($n = 8$). (**B**) There were no genotype differences in the investigation of a nonsocial odor ($n = 12$ long, $n = 8$ short allele). (**C**) Long-allele males ($n = 49$; $n = 45$ short allele) more quickly approached a novel juvenile animal, although there were no genotype differences in total frequency of investigation or duration of investigation in this task. (**D**) Long-allele males ($n = 32$) displayed increased probability of preferences for a familiar partner female over a novel stranger female. Short-allele males ($n = 30$) did not display a partner preference. In the table, a partner preference "PP" is defined as a partner preference score of 66.67% or greater, where the partner preference score = (partner contact time)/(partner contact time + stranger contact time). "EP" is an equal preference for either partner or stranger (33.33 to 66.66%). "SP" is a preference for the stranger (less than 33.33%). Both groups showed strong preferences for either partner or stranger (PP+SP versus EP) compared with a random probability distribution, but only the long-allele group showed a significant preference for partner (PP versus EP+SP), compared with a random probability distribution. Bars in (A, B, C, and D) represent means + SEM.

were randomly cross-fostered within 6 to 8 hours after birth to reduce the potential confounding variable of rearing environment. There were no genotype differences in birth rates (Mann-Whitney, $P > 0.05$); however, short-allele breeder pairs had higher rates of pup mortality (12 out of 25, versus 3 out of 36, $\chi^2 = 10.83$, $P < 0.001$). This effect was only apparent with the first litter, as both genotype groups had high rates of survival after the pairs had become experienced parents. Because paternal care in prairie voles is influenced by vasopressin, as well as selective V1aR antagonists (*21*), we measured parental care during the first ten post-natal days. Twenty hours of observations revealed no genotype differences in the amount of time the pair were on the nest together or in the time each parent was alone on the nest (Fig. 2A, Mann-Whitney, $P > 0.05$). Additionally, there were no genotype differences in the amount of time the nest was left unoccupied by both parents simultaneously (Fig. 2A, Mann-Whitney, $P > 0.05$). There were also no genotype differences in nursing postures adopted by the females (Fig. 2B, Mann-Whitney, $P > 0.05$). In contrast, there were significant genotype differences in the frequency of pup licking and grooming (Fig. 2C, Mann-Whitney, $P < 0.05$). This genotype difference can be fully attributed to a higher grooming frequency of long-allele males compared with short-allele males (Mann-Whitney, $P < 0.01$), as there were no genotype differences in pup licking and grooming frequency by the females (Mann-Whitney, $P > 0.05$). These data coincide with the prevailing hypothesis in the literature that vasopressin systems contribute to male, not female, species-typical behaviors. The genotype differences in total licking and grooming (15 to 20%) are only slightly less than the 20 to 30% differences in grooming rates in high versus low licking and grooming rat dams, which have well-established consequences for the receiving offspring (*22*).

Vasopressin and the V1aR have been implicated in social recognition and investigation processes in a variety of species (*23*). We had preliminary correlative evidence (*20*) from a random sample of 20 animals from our regular laboratory breeding colony to suggest that the microsatellite would highly predict levels of V1aR in the olfactory bulb, and that V1aR levels in the olfactory bulb would be associated with social behavior. It can be difficult to control the experiment-wise variability in social behavior tasks due to variation contributed by the stimulus animal. Therefore, as an initial test of social behavior, we tested the F_1 males for their behavior toward novel social odors. We placed each male in an empty arena for a 2-min acclimation period and then added a small cartridge filled with soiled bedding from the cage of an unrelated female of similar age. Long-allele males displayed a shorter latency to first approach, and higher frequency and longer duration of investigation compared with short-allele males (Fig. 3A, Mann-Whitney, $P < 0.05$). This effect appears to be specific to social odors, as there were no genotype differences in response to a banana-like odor, amyl-acetate (Fig. 3B, Mann-Whitney, $P > 0.05$).

To determine whether the behavioral differences observed in the social odor task would translate into differences in prosocial behavior toward another individual, a juvenile prairie vole (PND15 to 18) was placed in the test subject's home cage for 10 min, and the reaction of the test subject was recorded. As with the social odorant test just described, long-allele animals began investigating the juvenile with a reduced latency (Fig. 3C, Mann-Whitney, $P < 0.05$). There were no genotype differences in huddling or attack behavior, or in the frequency or duration of investigation measures as seen in the social odor task. Regardless, the genetic differences in approach latency have consequences for the probability of social contact in ethologically relevant contexts.

The partner preference test has been the hallmark assay for demonstrating a role for

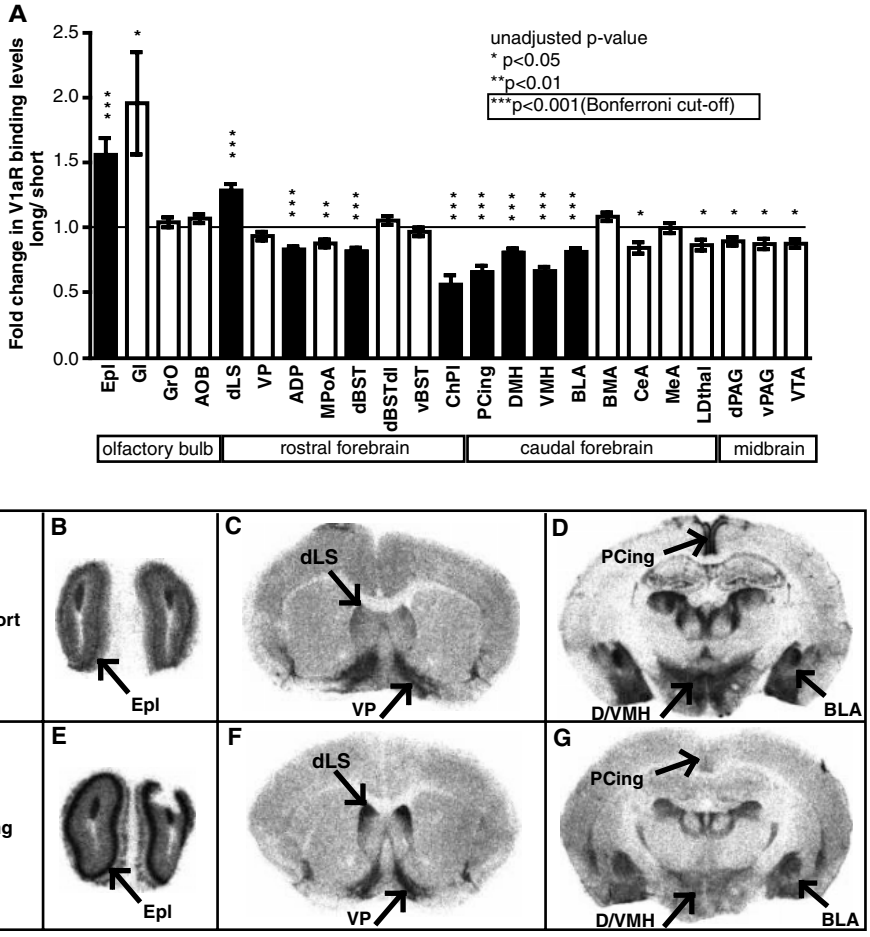


Fig. 4. Microsatellite length predicts robust differences in the distribution patterns of V1aR. (**A**) Compared with short-allele males ($n = 45$; line at unity), long-allele males ($n = 49$) have higher levels of V1aR in some brain regions and lower levels of V1aR in others. For each brain region, binding values were normalized to average values for the short-allele group. Bars represent means ± SEM of the long-allele males. (**B** to **D**) Examples of autoradiographs from short-allele males, showing lower levels of V1aR in the external plexiform layer of the olfactory bulb (B) and dorsal lateral septum (C), no differences in the ventral pallidum (C), and higher V1aR levels in the posterior cingulate cortex, hypothalamus, and amygdala (D). Matched sections of sample autoradiographs from long-allele males are displayed in (**E** to **G**). Abbreviations: ADP, anterior dorsal preoptic nucleus; AOB, accessory olfactory bulb; BLA, basolateral amygdala; BMA, basomedial amygdala; CeA, central amygdala; ChPl, choroid plexus; dBST, dorsal bed nucleus of the stria terminalis; dBSTdl, dorsolateral dBST; dLS, dorsal lateral septum; DMH, dorsomedial hypothalamus; dPAG, dorsal periaqueductal gray; D/VMH, DMH and VMH; EPl, external plexiform layer; Gl, glomerular layer; GrO, granular layer; LDthal, laterodorsal thalamus; MeA, medial amygdala; MPoA, medial preoptic area; PCing, posterior cingulate cortex; vBST, ventral BST; VMH, ventromedial hypothalamus; VP, ventral pallidum; vPAG, ventral PAG; VTA, ventral tegmental area.

vasopressin and V1aR in pair bonding. We paired the test males with sexually receptive females for an overnight cohabitation. We used a truncated (18-hour) cohabitation period in an effort to increase the variability in the development of partner preferences. In the 3-hour partner preference test, long- and short-allele males spent equal amounts of time in total social contact (student's t test, $P > 0.05$). However, long-allele males spent more time with their partner compared with the stranger, whereas short allele-males did not (Fig. 3D; two-way ANOVA, preference and genotype as factors, planned t test for partner time versus stranger time $P < 0.05$ in long-allele males, $P > 0.05$ in short-allele males). Criteria for a partner preference are met when the test animal spends twice as much social contact time with the partner compared with the stranger. As a group, long-allele males displayed partner preferences (Fig. 3D, $\chi^2 = 7.56$, $P < 0.05$), whereas short-allele animals did not ($\chi^2 = 2.4$, $P > 0.05$), when compared with a random distribution of preferences. Because there were no genotype differences in total social contact time, the genotype differences in partner preference behavior could be due to differences in some aspect of social discrimination of the two females. Perhaps

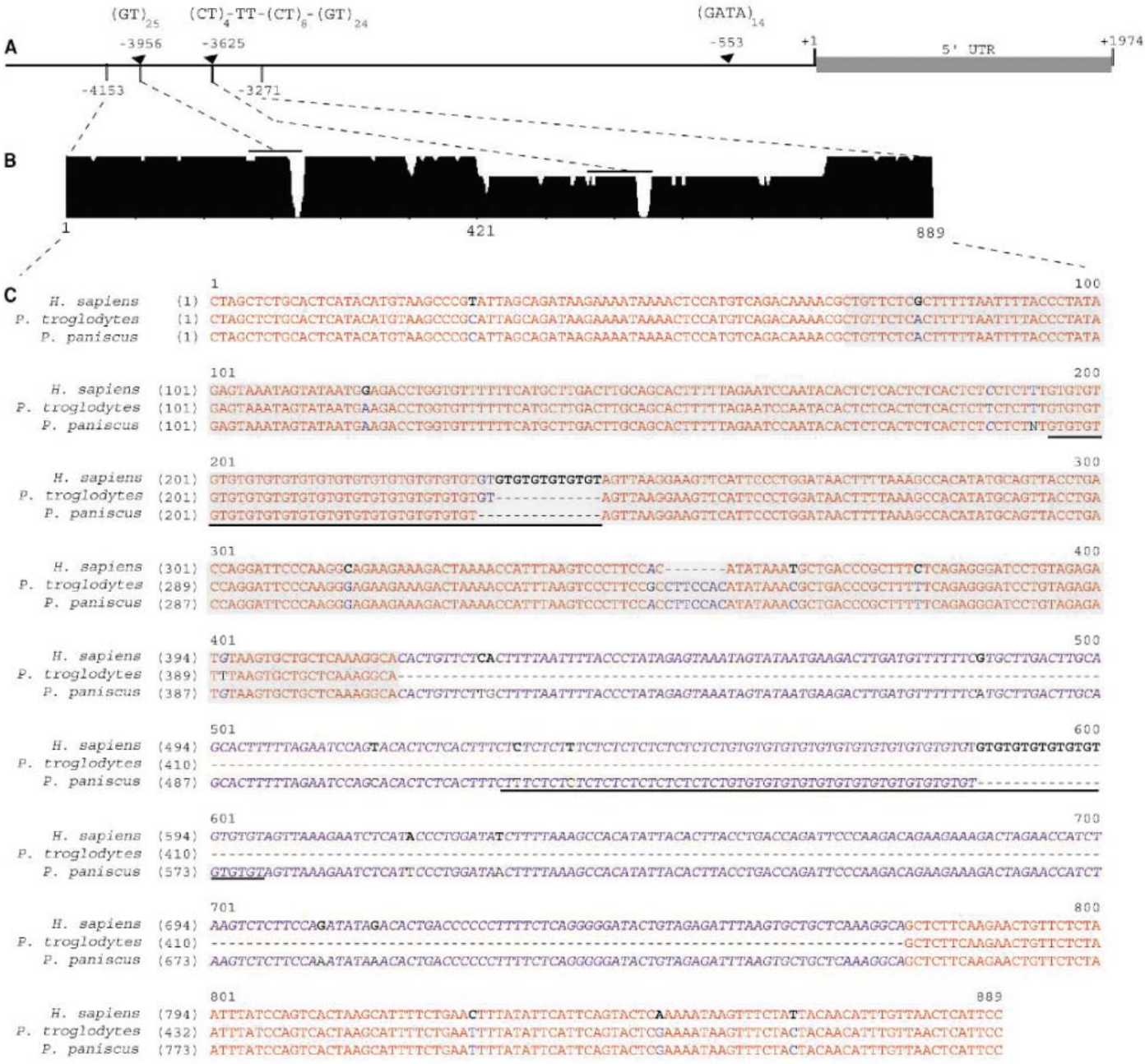


Fig. 5. Humans (*H. sapiens*) and bonobos (*P. paniscus*) share a cryptic microsatellite element in the 5′ regulatory region of *AVPR1A*, which is absent in the chimpanzee (*P. troglodytes*). The schematic (**A**) illustrates the position of 5′ microsatellite elements in relation to the annotated transcription start site (+1) of the human *AVPR1A* gene (start codon at +1974). Human, chimpanzee, and bonobo similarity plot (**B**) at this region reveals high levels of homology around the –3956 bp microsatellite, whereas the microsatellite at –3625 bp is not as conserved. The reduction in similarity around the –3625 bp microsatellite is due to the complete absence of ~360 bp in chimpanzees (**C**). Underscores indicate the positions of the two repeat regions. Red letters indicate sequence that is conserved across all three species. Blue sequence represents conservation in two out of three species; blue italicized sequence represents sequence that is shared between human and bonobo, but not chimpanzee. Bold black letters highlight sequence that is unique to humans. The bonobo/human-specific region appears to be a duplication of the preceding sequence, indicated by the shaded gray box.

short-allele males need a longer cohabitation period to learn to distinguish partner from stranger.

To control for the possibility of a confounding effect of V1aR on anxiety, we tested the animals in the elevated plus maze and the open field test. Although there were subtle genotype differences in each of these tests (see supporting online material), neither test revealed genotype differences on the classic measures of trait anxiety: namely, time in the open arms of the elevated plus maze and time in the center of the open field test. Therefore, although there may be subtle genotype differences in some measure of emotionality, there were clearly no genotype differences in the classic measures of trait anxiety to sufficiently explain the observed differences in social behavior.

The genotype differences in social behavior are associated with robust genotype differences in V1aR binding in the brains of the F_1 males (Fig. 4). Several brain regions showed up to 50% changes in the quantity of V1aR binding. In some brain regions, a long allele conferred a higher level of receptor binding (olfactory bulb, lateral septum), and in other regions a long allele conferred a lower level of receptor binding (amygdala, hypothalamus, posterior cingulate cortex). Microsatellites may generate phenotypic diversity by acting in a cell-type dependent manner, i.e., altering the pattern of gene expression across brain regions, rather than globally altering total levels of expression. This could generate heritable behavioral diversity by altering the neural circuits that are engaged in behavioral contexts that cause the central release of vasopressin.

Several of the brain regions with genotype differences in V1aR levels have previously been implicated in the behaviors described above. For example, V1aR activation in the lateral septum has been associated with both increased rates of pup licking and grooming, as well as increased partner preference formation (*21*, *24*). Our long-allele males showed higher levels of V1aR in the lateral septum, higher rates of pup licking and grooming, and higher levels of partner preference formation. The long-allele males also showed higher levels of V1aR in the external plexiform layer of the olfactory bulb. Lesions of the olfactory bulb inhibit partner preference formation and social approach behaviors in prairie voles (*25*, *26*). Also, pharmacological manipulation of vasopressin systems in both the olfactory bulb and lateral septum modulate social recognition in rats and mice (*27–29*). Therefore, genotype differences in V1aR binding in the lateral septum and olfactory bulb are likely candidates for serving as the neural substrates underlying the observed genotype differences in social behavior. Variation in the lateral septum and olfactory bulb probably influence the requisite social recognition component of pair bonding.

Previous comparative (*11*, *12*), pharmacological (*30*), and viral vector gene-transfer (*13*, *31*) experiments have demonstrated that species differences in V1aR levels in the ventral pallidum contribute to species differences in the development of partner preference formation in voles. Interestingly, V1aR levels in the ventral pallidum, which are associated with species differences in social structure, were not associated with genotype within the prairie vole species. Perhaps V1aR binding levels in the ventral pallidum are now genetically fixed by conservation of the 5′ end of the prairie-specific microsatellite, but the hypervariable 3′ end produces variation in other brain regions, such as the lateral septum, olfactory bulb, and hypothalamus, within this species.

Although we have established a clear functional role for the microsatellite in cell culture, the possibility still exists that some of the variation in V1aR binding patterns and behavior may be due to linkage disequilibrium with the microsatellite. However, the in vitro evidence in this manuscript combined with ample experimental evidence from other reports implicating V1aR in social behavior indicate that the microsatellite is likely a causal mechanism of behavioral trait variation through alterations in V1aR distribution.

We theorize that microsatellites in the regulatory regions of the *avpr1a* gene confer this locus with high levels of evolvability, which in itself may be a target of selection (*32*). Interestingly, four polymorphic microsatellites surround the human *avpr1a* gene (*33*). Two independent reports have indicated modest association of microsatellite alleles at the –3625 bp locus with autism (*34*, *35*), which is a disease of profound social deficit. Considering that variation at this locus may have important implications for our own species-typical social behavior, we compared the publicly available *avpr1a* gene sequences of chimpanzees [*Pan troglodytes* (*36*)] and humans (*37*) and found that 360 bp in and around this microsatellite locus was deleted in chimpanzees, although the flanking regions were >96% conserved. In contrast, the same locus in the *avpr1a* gene in the bonobo (*Pan paniscus*), which is known for its socio-sexual reciprocity and bonding (*38*), has high homology with the human microsatellite (Fig. 5). Perhaps in primate species, as in vole species, both inter- and intraspecific variation in regulatory microsatellites of the *avpr1a* gene can give rise to behavioral variation via altered regulation of the distribution of this gene product across individuals.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/308/5728/1630/DC1
Materials and Methods
Fig. S1

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Diversity of the Human Intestinal Microbial Flora

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The human endogenous intestinal microflora is an essential "organ" in providing nourishment, regulating epithelial development, and instructing innate immunity; yet, surprisingly, basic features remain poorly described. We examined 13,355 prokaryotic ribosomal RNA gene sequences from multiple colonic mucosal sites and feces of healthy subjects to improve our understanding of gut microbial diversity. A majority of the bacterial sequences corresponded to uncultivated species and novel microorganisms. We discovered significant intersubject variability and differences between stool and mucosa community composition. Characterization of this immensely diverse ecosystem is the first step in elucidating its role in health and disease.

The endogenous gastrointestinal microbial flora plays a fundamentally important role in health and disease, yet this ecosystem remains incompletely characterized and its diversity poorly defined (*1*). Critical functions of the commensal flora include protection against epithelial cell injury (*2*), regulation of host fat storage (*3*), and stimulation of intestinal angiogenesis (*4*). Because of the insensitivity of cultivation, investigators have begun to explore this ecosystem using molecular fingerprinting methods (*5*) and sequence analysis of cloned microbial small-subunit ribosomal RNA genes [16S ribosomal DNA (rDNA)] (*6*–*9*). However, such studies have been limited by the relative paucity of sequenced gene fragments, the use of fecal biota as a surrogate for the entire gut microflora, and little attention given to potential differences between specific anatomical sites. In addition, variation associated with time, diet, and health status have not been adequately described, nor have the relative importance and contributions of each source (*10*).

Surface-adherent and luminal microbial populations may be distinct and may fulfill different roles within the ecosystem. For example, the biofilm-like architecture of the mucosal microbiota, in close contact with the underlying gut epithelium, facilitates beneficial functions including nutrient exchange and induction of host innate immunity (*11*). Fecal samples are often used to investigate the intestinal microflora because they are easily collected. However, the degree to which composition and function of the fecal microflora differ from mucosal microflora remains unclear. We undertook a large-scale comparative analysis of 16S rDNA sequences to characterize better the adherent mucosal and fecal microbial communities and to examine how these microbial communities differed between subjects and between mucosal sites.

Mucosal tissue and fecal samples were obtained from three healthy adult subjects (A, B, and C) who were part of a larger population-based case-control study (table S1) (*12*). Mucosal samples were obtained during colonoscopy from healthy-appearing sites within the six major subdivisions of the human colon: cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum. Fecal samples were collected from each subject 1 month following colonoscopy (*12*). We focused on 16S rDNA given its universal distribution among all prokaryotes, the presence of diverse species-specific domains, and its reliability for inferring phylogenetic relationships (*13*). The 16S rDNA was amplified from samples with polymerase chain reaction (PCR) and broad-range bacterial and archaeal primers (*12*). The 7 samples from subject B and the fecal sample from subject C yielded archaeal products; all 21 samples yielded bacterial products. PCR products were cloned and sequenced bidirectionally, and numerical ecology approaches were applied.

Initially, a phylotype census was performed on each sample (table S2). A total of 11,831 bacterial and 1524 archaeal near-full-length, nonchimeric 16S rDNA sequences were subjected to phylogenetic analysis. Using 99% minimum similarity as the threshold for any pair of sequences in a phylotype (or operational taxonomic unit) as calculated by dissimilarity matrices and the DOTUR program (*12*), we identified a total of 395 bacterial phylotypes (Fig. 1). In contrast, all 1524 archaeal sequences belonged to a single phylotype (*Methanobrevibacter smithii*); these archaeal sequences were excluded from further analyses. This remarkable apparent difference in diversity of the two prokaryotic domains in the gut was reminiscent of results from soil and ocean (*14*).

Of the 395 bacterial phylotypes, 244 (62%) were novel (table S3), and 80% represented sequences from species that have not been cultivated (*12*). Most of the inferred organisms were members of the *Firmicutes* and *Bacteroidetes* phyla (Fig. 1 and fig. S1), which is concordant with other molecular analyses of the gut flora (*6*, *7*, *9*). The *Firmicutes* phylum consisted of 301 phylotypes, 191 of which were novel; most (95%) of the *Firmicutes* sequences were members of the *Clostridia* class. We detected a substantial number of Firmicutes related to known butyrate-producing bacteria (2454 sequences, 42 phylotypes) (*15*, *16*), all of which are members of clostridial clusters IV, XIVa, and XVI. We expected prominent representation of this functional group among our healthy control subjects, given its role in the maintenance and protection of the normal colonic epithelium (*16*). Large variations among the 65 *Bacteroidetes* phylotypes were noted between subjects (Fig. 1), as described previously (*6*, *7*). *B. thetaiotaomicron* was detected in each subject and is known to be involved in beneficial functions, including nutrient absorption and epithelial cell maturation and maintenance (*17*). Relatively few sequences were associated with the *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia* phyla (fig. S1). The low abundance of *Proteobacteria* sequences (including *Escherichia coli*) was not surprising, given that facultative species may represent ~0.1% of the bacteria in the strict anaerobic environment of the colon; this is consistent with previous findings (*6*, *8*, *9*). Three sequences from two subjects (represented by AY916143) clustered with unclassified sequences previously identified from mammalian gut samples. These sequences appear to represent a novel lineage, deeply branching from the *Cyanobacteria* phylum and chloroplast sequences.

No complex microbial community in nature has been sampled to completion. In addition to its biases and inability to distinguish live from dead organisms, the limited sensitivity of broad-range PCR may hinder detection of rare phylotypes. We used several nonparametric methods to explore the diversity and coverage of our clone libraries. Phylotype richness estimations suggested that at least 500 phylotypes would be detected with

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continued sequencing from our samples (≥130, ≥300, and ≥200 phylotypes in subjects A, B, and C) (Fig. 2 and figs. S2 and S3). These estimates must be considered as lower bounds, because both the observed and the estimated richness have increased in parallel with additional sampling effort (Fig. 2 and fig. S3). Coverage was 99.0% over all bacterial clone libraries combined, meaning that one new unique phylotype would be expected for every 100 additional sequenced clones (*18*).

The microbial community appeared more diverse in subject B than in A or C, based on inspection of the richness and evenness of the clone distribution across the phylogenetic tree (Fig. 1). The Rao diversity coefficient (*19*), which accounts for both phylotype abundance and dissimilarity, was indeed higher for B than for the other subjects (fig. S7). This pattern was not found with traditional, that is, Shannon and Simpson, diversity indices, which assess only relative phylotype abundance (*20*). Within each subject, the mucosal samples demonstrated similar diversity profiles, regardless of the index used (fig. S7).

Previous investigations have not rigorously addressed possible differences in the intestinal microflora between subjects, between anatomical sites, or between stool and mucosal communities. We applied techniques that are based on the relative abundance of sequences within communities and the extent of genetic divergence between sequences. We first compared inter- and intrasubject variability using double principal coordinate analysis (DPCoA) (*19*). The greatest amount of variability was explained by intersubject differences; stool-mucosa differences explained most of the variability remaining in the data (Fig. 3). The relative lack of variation among mucosal sites was further examined. The F_{ST} statistic of population genetics (*21*) was used to compare genetic diversity within each subject; this revealed that the mucosal populations of subjects A and B were significantly distinct com-

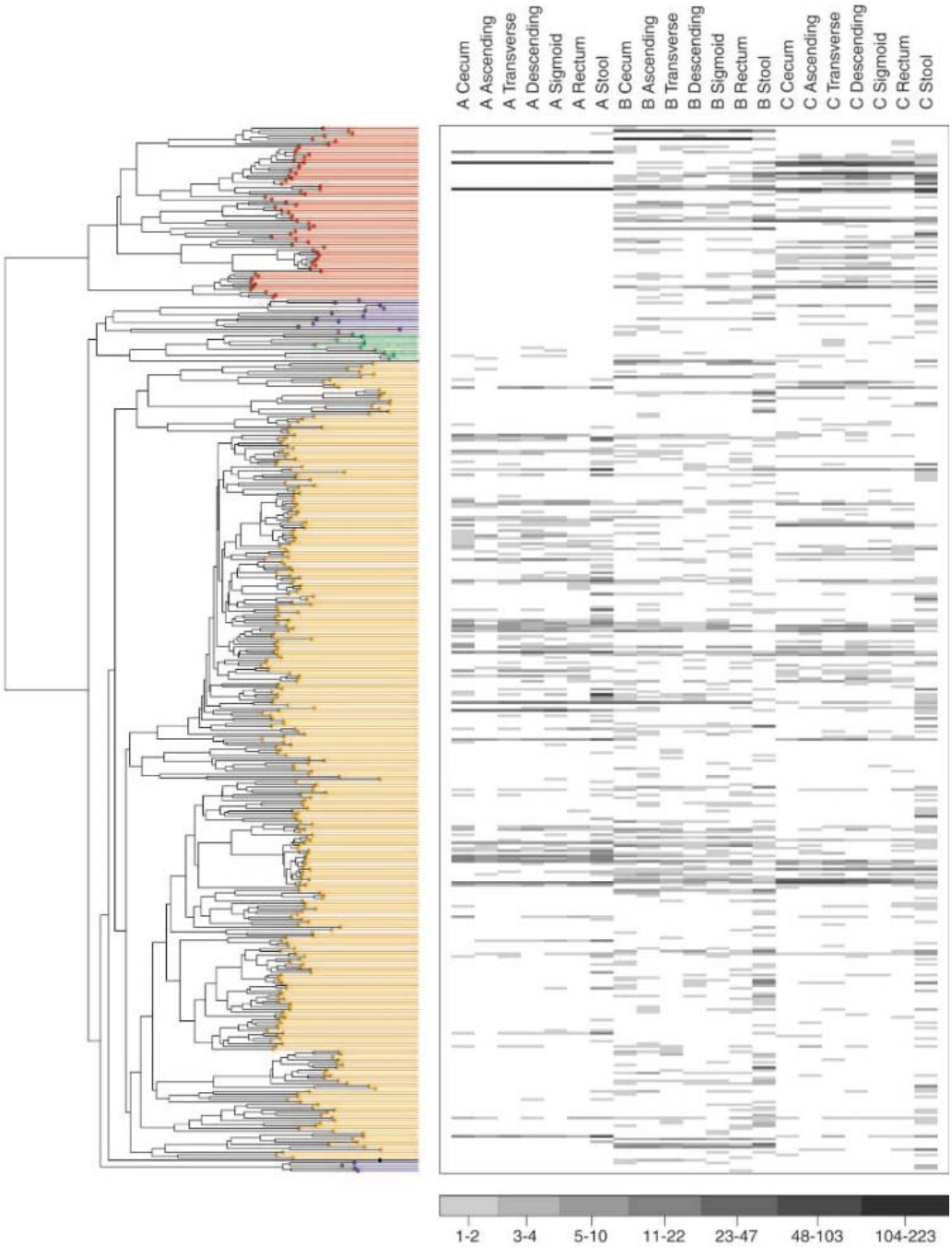

Fig. 1. Number of sequences per phylotype for each sample. The *y* axis is a neighbor-joining phylogenetic tree containing one representative of each of the 395 phylotypes from this study; each row is a different phylotype. The phyla (*Bacteroidetes*, *non-Alphaproteobacteria*, unclassified near *Cyanobacteria*, *Actinobacteria*, *Firmicutes*, *Fusobacteria*, and *Alphaproteinobacteria*, ordered top to bottom) are color coded as in Fig. 3 and fig. S1. Each column is labeled by subject (A, B, C) and anatomical site. For each phylotype, the clone abundance is indicated by a grayscale value.

pared with the overall mucosal diversity (table S5). However, in both of these subjects, a single mucosal library had a deviant genetic diversity index; exclusion of this library from the analysis led to an insignificant F_{ST} statistic in each case (*12*). Taken as a whole, these results confirmed little genetic variation among subject-specific mucosal libraries.

We then asked whether nonrandom distributions of phylogenetic lineages accounted for any variation among all samples. Using a modification of the phylogenetic (P) test (*12*, *21*), we found that stool and pooled mucosal libraries harbored distinct lineages ($P < 0.001$) (table S5); however, distinct lineages were not found among the individual mucosal libraries. We sought further anatomic precision in explaining library distinctions using the ∫-LIBSHUFF program (*22*). We found that mucosal clone libraries were similar to the other mucosal libraries from the same subject, with two exceptions (fig. S6). The library from the ascending colon of subject A was a subset of every other mucosal population from that subject (P values < 0.0017), and the descending colon library from subject B was a subset of the ascending colon library in that subject ($P = 0.0005$). Such inconsistencies among mucosal subpopulations suggested a pattern of patchiness in the distribution of mucosal bacteria rather than a homogenous gradient along the longitudinal axis of the colon. ∫-LIBSHUFF also revealed that nearly all mucosal libraries from subjects B and C were significantly distinct from the corresponding stool library, whereas each mucosal library from subject A was a subset of the stool library. We postulate that the fecal microbiota represents a combination of shed mucosal bacteria and a separate nonadherent luminal population; however, these data must be interpreted with caution, given the delay between stool and mucosa sampling.

Bacterial diversity within the human colon and feces is greater than previously described, and most of it is novel. Differences between individuals were significantly greater than intrasubject differences, with the exception of variation between stool and adherent mucosal communities. Complicating this picture is our evidence for patchiness and heterogeneity. This patchiness did not display an obvious pattern along the course of the colon but may reflect microanatomic niches. Given that each mucosal sample contained a similar distribution of organisms within higher order taxa (Fig. 1), the variation we

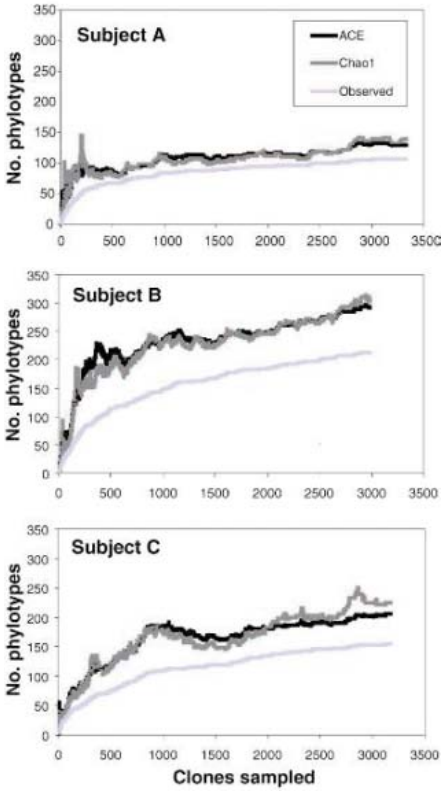

Fig. 2. Collector's curves of observed and estimated phylotype richness of pooled mucosal samples per subject. Each curve reflects the series of observed or estimated richness values obtained as clones are added to the data set in an arbitrary order. The curves rise less steeply as an increasing proportion of phylotypes have been encountered, but novel phylotypes continue to be identified to the end of sampling. The relatively constant estimates of the number of unobserved phylotypes in each subject as observed richness increases (the gap between observed and estimated richness) indicate that estimated richness is likely to increase further with additional sampling. The Chao1 estimator and the abundance-based coverage estimator (ACE) are similar, but the ACE is less volatile because it uses more information from the abundance distribution of observed phylotypes. Individual-based rarefaction curves are depicted in figs. S4 to S6.

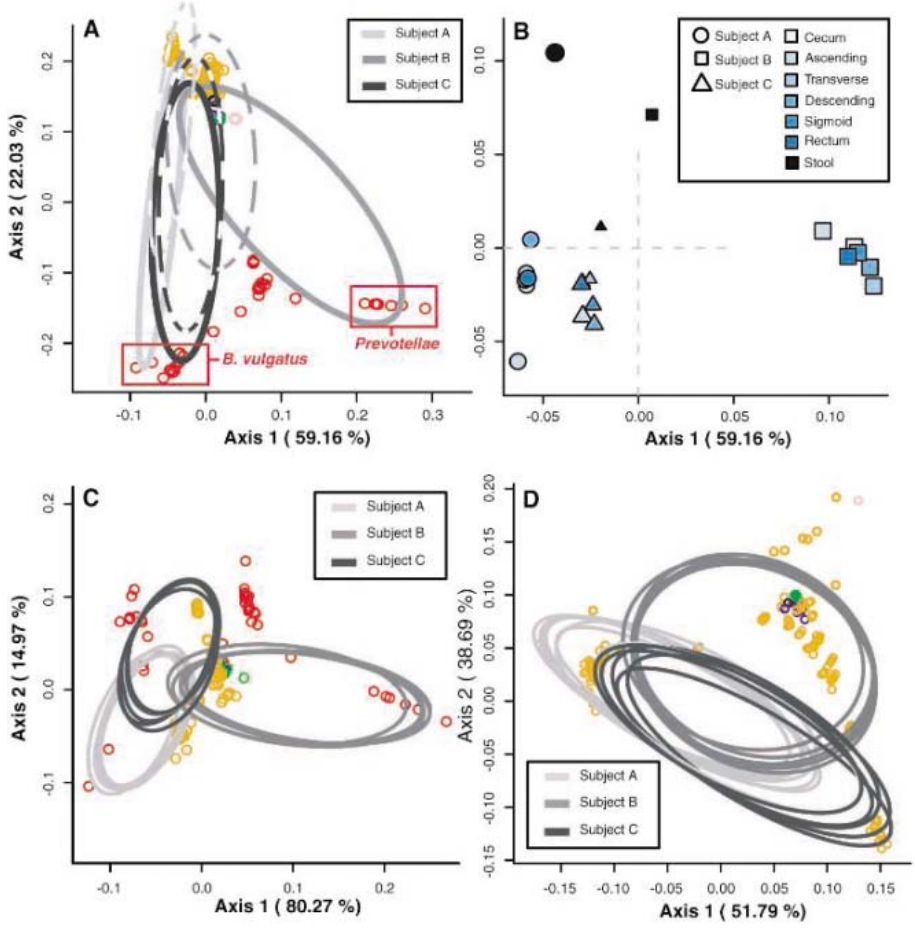

Fig. 3. DPCoA for (**A**) colonic mucosa (solid lines) and stool (dashed lines), (**C**) colonic mucosal sites alone, and (**D**) mucosal sites excluding *Bacteroidetes* phylotypes. Phylotypes are represented as open circles, colored according to phylum as in Fig. 1. Phylotype points are positioned in multidimensional space according to the square root of the distances between them. Ellipses indicate the distribution of phylotypes per sample site, except in (A), where all mucosal sites are represented by one ellipse. Percentages shown along the axes represent the proportion of total Rao dissimilarity captured by that axis. (A) is the best possible two-dimensional representation of the Rao dissimilarities between all samples (*12*). (**B**) is an enlarged view of (A), depicting the centroids of each site-specific ellipse. Subject ellipse distributions remain distinct after stool phylotypes (C) and *Bacteroidetes* phylotypes (D) are excluded from the analysis.

observed at the genus or species level may be the result of colonization resistance by the more abundant members within similar functional groups (*23*). Whether the gut microbiota undergoes such nonrandom assembly remains unclear.

Ecological statistical approaches reveal previously unrecognized irregularities in the architecture of complex microbial communities. High-resolution spatial, temporal, and functional analyses of the adherent human intestinal microbiota are still needed. In addition, the effects of host genetics and of perturbations such as immunosuppression, antimicrobials, and change in diet have yet to be carefully defined. We anticipate that microarrays, single-cell analysis, and metagenomics [e.g., a "Second Human Genome Project" (*24*)] will complement the approach we have illustrated and hasten our understanding of human-associated microbial ecosystems.

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Supporting Online Material

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Fungal Pathogen Reduces Potential for Malaria Transmission

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Using a rodent malaria model, we found that exposure to surfaces treated with fungal entomopathogens following an infectious blood meal reduced the number of mosquitoes able to transmit malaria by a factor of about 80. Fungal infection, achieved through contact with both solid surfaces and netting for durations well within the typical post-feed resting periods, was sufficient to cause >90% mortality. Daily mortality rates escalated dramatically around the time of sporozoite maturation, and infected mosquitoes showed reduced propensity to blood feed. Residual sprays of fungal biopesticides might replace or supplement chemical insecticides for malaria control, particularly in areas of high insecticide resistance.

The use of pyrethroid insecticides on bednets or on walls and ceilings is the mainstay of malaria vector control. However, some forms of resistance are a threat to the long-term effectiveness of such measures (*1*, *2*). With practical implementation of novel molecular interventions still years off (*3*–*6*), there is a pressing need for practical alternatives for malaria control (*7*).

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Several studies have investigated the use of microbial agents for biological control of mosquitoes (see *8*–*10* for reviews). The most successful approach has been the use of microbial biopesticides, such as *Bacillus thuringiensis*, for control of the larval stages (*8*, *9*). Here, we report the potential of fungal entomopathogens for indoor use against adult mosquitoes to reduce malaria transmission. Oil-based formulations of fungal entomopathogens are a relatively recent innovation that enables economically viable spore application in ultralow-volume sprays under a wide range of environmental conditions (*11*, *12*). These formulations create an opportunity to apply fungal pathogens for use on indoor surfaces of houses or curtains where some malaria vector species rest after a blood meal or on bednets to which mosquitoes are attracted by the odor of the occupant. Fungal entomopathogens infect through external contact, with spores germinating and penetrating through the cuticle before proliferating in the hemocoel. Natural mosquito-mosquito transmission is unlikely, because this would require contact between an uninfected adult and a sporulating cadaver. In other pest-control contexts, contact with fungal spores in a spray residue has proved to be a highly efficient means of infecting insects (*13*, *14*) because it does not rely on direct hit of the target and enables accumulation of high doses of pathogen over time through continued or repeat exposure.

Our experimental system comprised *Anopheles stephensi* and the rodent malaria *Plasmodium chabaudi*. Our first experiment was a basic mortality screen of eight Hyphomycetes fungal isolates from two common species, *Beauveria bassiana* and *Metarhizium anisopliae*. The specific isolates were selected based on their known biological activity, that is, either known generalists or those originally isolated from dipteran hosts (*15*). The basic assay technique exposed blood-fed adult female mosquitoes to oil-based spray residues inside replicated cardboard pots (*16*). Mosquitoes were introduced 24 hours after the pots were sprayed and, for this initial screen, remained in the sprayed pots for 14 days. The eight fungal isolates varied in virulence to *A. stephensi* (Fig. 1 and table S2), six of which produced >80% mortality by day 14, with >70% of the cadavers bearing sporulated fungi. High mortality by day 14 is encouraging because that is about the time taken for *Plasmodium* to develop from ingested gametocytes to infective sporozoites.

After initial screening, we selected the *B. bassiana* isolate IMI 391510 for further evaluation. Although it was not the most virulent isolate of those tested, this isolate is used in an existing agricultural biopesticide product, and the provision of a registration dossier would accelerate progress to field-scale evaluation.

We assessed the impact of IMI 391510 by direct topical application against *A. stephensi* and as a spray residue on cage mesh, partially simulating treatment of bednets (but with a longer exposure than would occur with free-flying mosquitoes approaching an occupied bednet). The treatments killed 91% and 93% of mosquitoes, respectively, by day 14. This compared with 38% mortality in the control mosquitoes sprayed with oil only. Significant differences in median survival times were seen between treatments: controls, >14 days; topical application, 8.0 days; treated netting, 7.0 days (log-rank statistic = 5.43, $P = 0.02$).

Anopheline mosquitoes tend to rest on structures such as walls and ceilings for less than 24 hours after a blood meal (*17*, *18*). Thus, we simulated short-term exposures after repeat blood feeds by exposing adult *A. stephensi* to the fungal spray residue for various combinations of 6 hours at 3-day intervals [3 days representing the approximate length of the feeding cycle (*19*)]. No difference was detected between any of the various control batches at the end of the assessment period (table S3, $F_{4,10} = 0.24$, $P = 0.91$). For the fungal treatments, there was a significant difference in survival between exposure regimes 14 days after their first exposure ($F_{4,10} = 6.0$, $P = 0.01$), although this was entirely due to the more rapid mortality of the day 0 regime compared with the day 12 regime (Tukey HSD, $P = 0.005$). No other comparisons revealed significant differences. Thus, limiting the time that mosquitoes were exposed to the spray residue did not alter overall survival compared with lifetime exposure shown in Fig. 1. For example, the day 12 treatment, with just one 6-hour exposure to a 12-day-old spray residue, showed 89% mortality 14 days after exposure (Fig. 2).

To assess the potential of this biopesticide to reduce malaria transmission, we tested *A. stephensi* infected with the CW clone of the rodent malaria *Plasmodium chabaudi* (*16*) (Malaria), compared with uninfected controls (Control), a *B. bassiana* only treatment (*Beauveria*), and a treatment combining *P. chabaudi* and *B. bassiana* (*Beauveria*+Malaria). Subsamples of mosquitoes were dissected to estimate *Plasmodium* prevalence at the oocyst (day 7) and sporozoite (day 14) stages.

Median survival times for the Control and Malaria treatments were not significantly different from one another (both treatments >14 days; log-rank statistic = 0.06, $P = 0.81$), nor was there a difference between the two fungal treatments (*Beauveria* and *Beauveria*+Malaria both 9.0 days; log-rank statistic = 0.34, $P = 0.56$). By day 14, 90% of the mosquitoes in the *Beauveria* treatment had died, with 95% mortality in the *Beauveria*+Malaria treatment (Fig. 3A). From day 11, there was a marked escalation in the mortality rate in the *Beauveria*+Malaria treatment, such that by day 14, daily mortality rate was about 65 times as high as that for the malaria-only treatment (Fig. 3B).

There were no differences in the concentrations of gametocytes in mice fed the two malaria treatments ($F_{1,11} = 0.19$, $P = 0.67$).

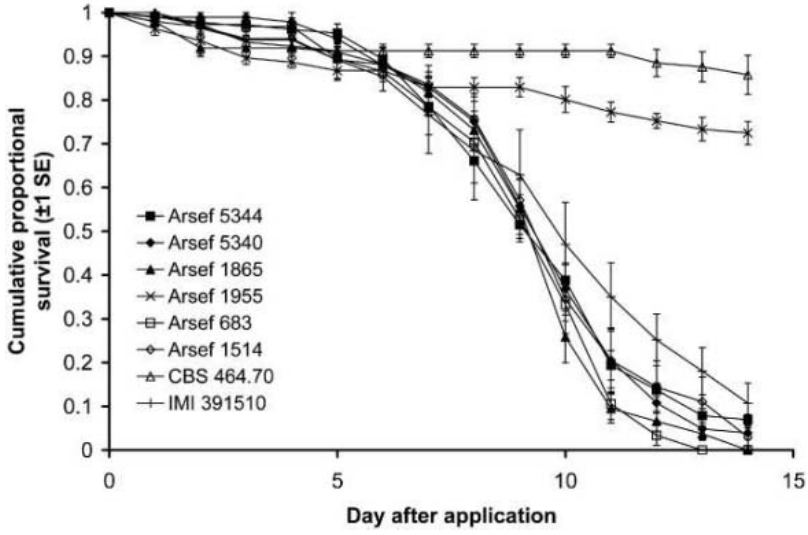

Fig. 1. Cumulative proportional survival of adult *A. stephensi* after exposure to oil-based spray residues containing spores of different fungal entomopathogens. Data represent means ± SEM from four replicates, each containing 40 female *A. stephensi*. Details on the fungal isolates are provided in (*16*).

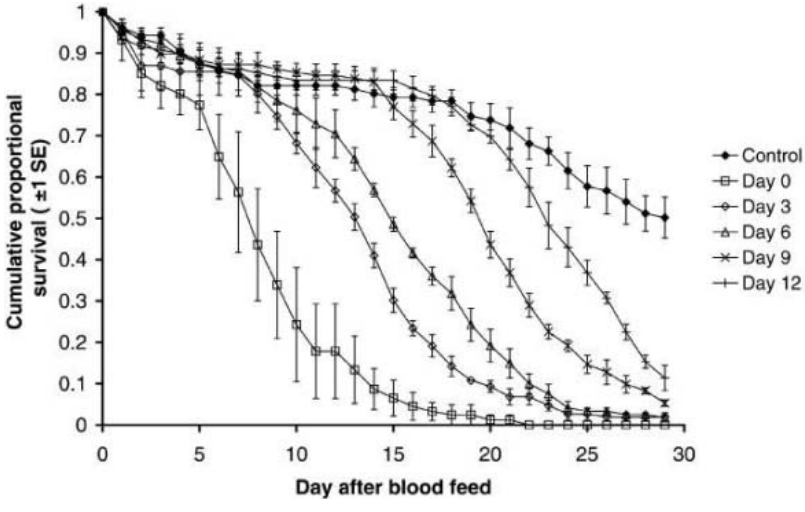

Fig. 2. Cumulative proportional survival of adult *A. stephensi* after exposure to oil-based spray residues containing the fungal pathogen *B. bassiana*, isolate IMI 391510. The lines indicate temporal exposure treatments. For the day 0 treatment, blood-fed *A. stephensi* were introduced to pots sprayed with *B. bassiana* for 6 hours and then removed to an unsprayed pot. On day 3, these mosquitoes were returned to the original sprayed pot for a further 6 hours and then removed again. This continued every three days until day 12. The remaining treatments were staggered such that the day 3 treatments were not introduced to a sprayed pot until day 3 and were subsequently exposed every 3 days for 6 hours until day 12. Days 6, 9, and 12 treatments were first introduced to sprayed pots on those days, and subsequently every 3 days as above. The day 12 treatment was, therefore, exposed only once, for 6 hours, to a pot sprayed 12 days previously. Matching controls for these treatments were moved between blank oil-sprayed pots and unsprayed pots at equivalent frequencies and exposure periods. Data for the respective treatments represent means ± SEM from four replicates, each containing ~40 female *A. stephensi*. The control line shows the control data for the day 0 exposure treatment (other control lines have been omitted for clarity because there were no significant differences between control treatments).

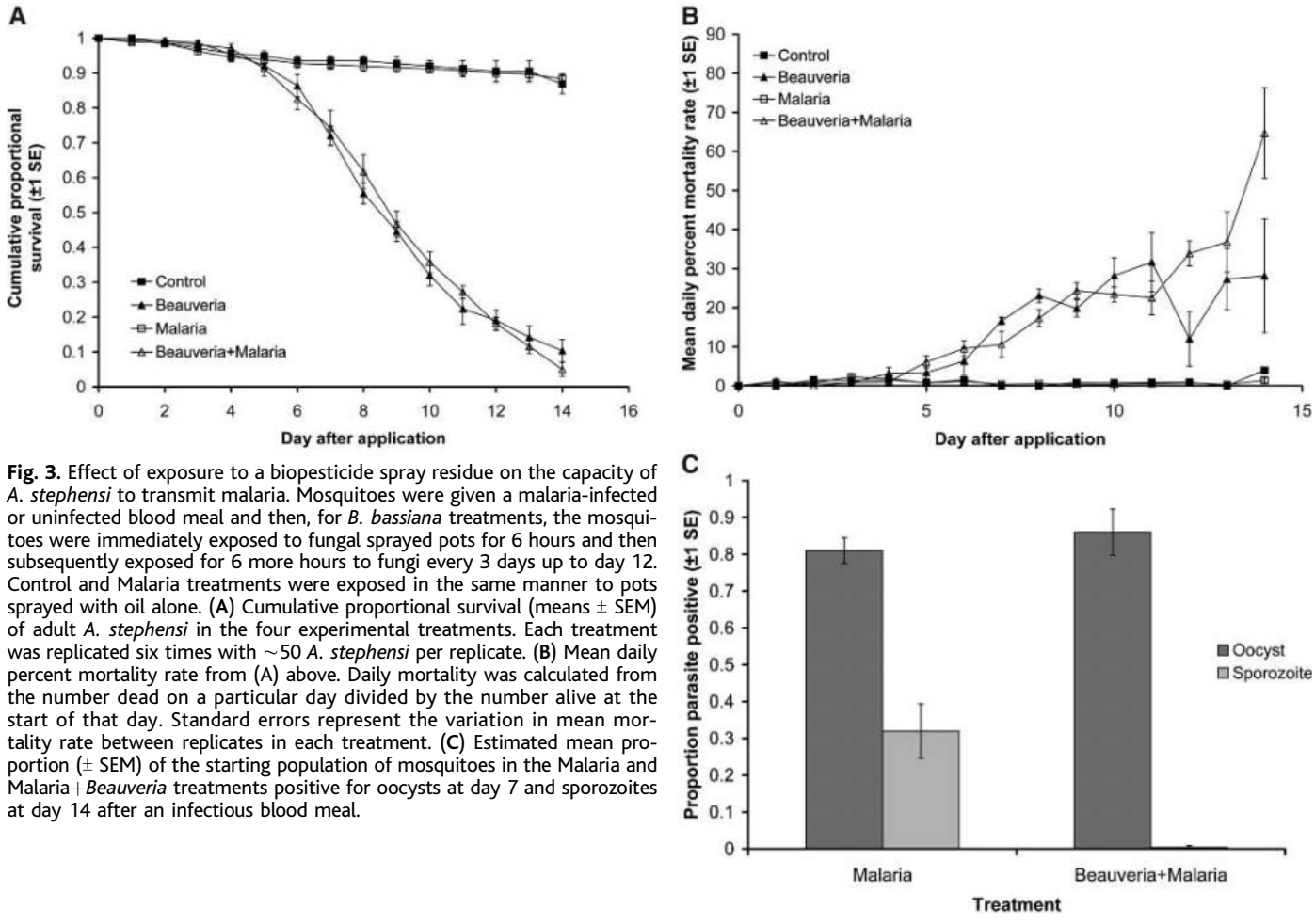

Fig. 3. Effect of exposure to a biopesticide spray residue on the capacity of *A. stephensi* to transmit malaria. Mosquitoes were given a malaria-infected or uninfected blood meal and then, for *B. bassiana* treatments, the mosquitoes were immediately exposed to fungal sprayed pots for 6 hours and then subsequently exposed for 6 more hours to fungi every 3 days up to day 12. Control and Malaria treatments were exposed in the same manner to pots sprayed with oil alone. (**A**) Cumulative proportional survival (means ± SEM) of adult *A. stephensi* in the four experimental treatments. Each treatment was replicated six times with ~50 *A. stephensi* per replicate. (**B**) Mean daily percent mortality rate from (A) above. Daily mortality was calculated from the number dead on a particular day divided by the number alive at the start of that day. Standard errors represent the variation in mean mortality rate between replicates in each treatment. (**C**) Estimated mean proportion (± SEM) of the starting population of mosquitoes in the Malaria and Malaria+*Beauveria* treatments positive for oocysts at day 7 and sporozoites at day 14 after an infectious blood meal.

Similarly, oocyst prevalence (Fig. 3C) and mean number of oocysts per midgut did not differ ($F_{1,11} = 0.55$, $P = 0.46$, and $F_{1,11} = 2.35$, $P = 0.16$, respectively). However, on day 14, 35% (109/310) of the mosquitoes remaining in the Malaria treatment were found to be sporozoite positive, compared with only 8% (1/12) in the *Beauveria*+Malaria treatment. Taking into account the difference in survival to day 14 of these two groups (Fig. 3A), this equates to 31% (109/352) of the initial mosquitoes in the Malaria treatment group being alive and potentially able to transmit at day 14, compared with only 0.4% (1/256) in the *Beauveria*+Malaria treatment (Fishers exact test, $P < 0.001$). Thus, fungal exposure led to a reduction of transmission risk by a factor of about 80. This result is supported by a further experiment with a different *P. chabaudi* clone (*20*).

Our results demonstrate the potential of oil-based formulations of fungal entomopathogens to reduce malaria transmission by reducing adult mosquito survival and altering *Plasmodium* survival/maturation in the mosquitoes. In addition, we have found that between days 8 and 14, fungal infection interferes with the ability of *A. stephensi* to take a blood meal, likely reducing transmission potential still further (*20*, *21*). Mosquitoes can be infected by direct contact with spray droplets or by contact with spray residues on treated surfaces and netting, with 6 hours exposure sufficient to cause high levels of infection; this is well within the post–blood feed resting period for the majority of *Anopheles* malaria vector species (*17*, *18*). We identified a range of isolates causing ≥ 90% mortality within 12 to 15 days (*22*–*24*). Further screening is likely to reveal more virulent isolates, but it is also likely that vector survival in the wild is less than under laboratory conditions (*22*). Furthermore, mosquitoes cannot transmit sporozoites until about 2 weeks after an infectious blood feed, and rapid killing of the mosquito is not necessary for reducing malaria transmission. As emphasized by MacDonald (*25*) in justifying the possibility of malaria eradication by indoor residual spraying of insecticide, even limited changes in daily probability of survival of mosquitoes can have a large impact on the prospects of survival through the time required for *Plasmodium* maturation to the infective stage; hence, the substantial reduction in sporozoite-positive mosquitoes and the escalating daily mortality rate that we observe due to fungal infection are highly significant in terms of malaria control.

Fungal biopesticides are already registered for agricultural use alongside chemical insecticides in a number of African countries (*12*, *26*). Transferring use to mosquito targets could (subject to further safety testing and appropriate registration) be rapid, with biopesticide products slotting readily into existing chemical application methodologies and strategies (*22*). As part of an integrated strategy, they could be used to respond to, or avert, the emergence of serious levels of insecticide resistance. Development of resistance against fungal pathogens has not been reported for insects, but even if resistance does occur, cross-resistance with chemical insecticides seems extremely unlikely. In the longer term, there would seem to be additional promise for using residual sprays of fungal pathogens for novel paratransgenic approaches to deliver toxins or effector molecules that block sporogony within the vector (e.g., see *27*, *28*). Unlike malaria control by genetic modification of mosquitoes, the fitness of biopesticide transgenes could be quite low and, because secondary transfer of fungi from

mosquitoes is very unlikely, fungal transgenes would be much easier to control than mosquito transgenes.

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Supporting Online Material

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Materials and Methods
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An Entomopathogenic Fungus for Control of Adult African Malaria Mosquitoes

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Biological control of malaria mosquitoes in Africa has rarely been used in vector control programs. Recent developments in this field show that certain fungi are virulent to adult *Anopheles* mosquitoes. Practical delivery of an entomopathogenic fungus that infected and killed adult *Anopheles gambiae*, Africa's main malaria vector, was achieved in rural African village houses. An entomological inoculation rate model suggests that implementation of this vector control method, even at the observed moderate coverage during a field study in Tanzania, would significantly reduce malaria transmission intensity.

Mosquito vector control is an integral part of controlling malaria (*1*). In Africa, this is almost exclusively based on the use of chemical insecticides for indoor residual spraying and impregnation of bednets for killing adult mosquitoes (*2*–*6*). The high efficacy achieved at modest coverages results from the exquisite sensitivity of malaria transmission intensity to the daily survival rate of adult mosquitoes (*7*). However, the continuing use of both public health and agricultural insecticides has led to a substantial increase in physiological resistance of mosquitoes in recent years (*8*, *9*). These problems have increased interest in alternative and integrated implementation of vector control methods that include biological control. Although several effective biological larvicides exist (*10*), there have been no biological control agents effective against adult mosquitoes. Addressing this gap, we have recently reported encouraging results with entomopathogenic fungi from the Hyphomycetes (Imperfect Fungi) infecting and killing adults of the African malaria vector *Anopheles gambiae sensu stricto* through tarsal contact in laboratory containers (*11*, *12*). Unlike other mosquitocidal biocontrol agents, such as bacteria, microsporidia, and viruses, these fungi can infect and kill insects without being ingested. Tarsal contact alone is enough to kill the insect, a characteristic shared with insecticidal chemicals. Moreover, Hyphomycetous insect-pathogenic fungi, such as *Metarhizium anisopliae* and *Beauveria bassiana*, are produced commercially and used against several agricultural insect pests worldwide (*13*).

Here, we report the results of a field study in a rural village in Tanzania in which we assessed whether wild mosquitoes became infected and had reduced life spans after resting on 3 m^2 *M. anisopliae*–impregnated black (*14*) cotton sheets ("targets") suspended from ceilings in traditional houses (fig. S1). Pre- and postintervention mosquitoes were collected, and equal numbers of untreated and treated houses were included (*15*).

In the 10 study houses, we collected a total of 2939 mosquitoes, 1052 during the preintervention (3 weeks) and 1887 during the intervention period (3 weeks). These were maintained on a 10% glucose diet in paper cups until death, after which fungal infections were detected, retrospectively, by observation of emerging hyphae from mosquito cadavers (*16*). We found that 88.9% were *A. gambiae s.l.* (*17*) and 10.7% *Culex quinquefasciatus*. Overall, 53.6% of the mosquitoes were caught on the targets, and 46.4% elsewhere in the rooms (*18*). None of the mosquitoes that had been collected during the preintervention period, nor any of the mosquitoes collected from the control houses during the entire experimental study period were found to be infected with the fungus. Of the 580 female *A. gambiae s.l.* that were collected in the five treatment houses during the intervention period, 132 were infected with *M. anisopliae*.

There was no significant difference in longevity between mosquitoes that were collected before and uninfected mosquitoes that were caught after the intervention ($F = 2.903$, $P = 0.088$). Similarly, longevity of mosquitoes caught in the control houses was not different from that of noninfected mosquitoes collected in the treatment houses during the intervention period ($F = 0.91$, $P = 0.3411$). By contrast, fungus-infected *A. gambiae s.l.* had significantly shorter life spans compared with those of noninfected mosquitoes (Fig. 1; overall effect pooling both sexes, $F = 178.9$, $P < 0.001$). Median lethal times (LT_{50}) values were 3.70 and 3.49 days for *M. anisopliae*–infected males and females, respectively, and 5.88 and 9.30 days for uninfected males and females, respectively. Of the 188 infected

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mosquitoes, most were caught in the first 2 weeks after the start of the intervention; 80, 79, and 29 in the first, second, and third week, respectively. This decline in infectivity was consistent with results of the conidial viability checks during the intervention period. Although conidia that were kept in suspension barely lost viability (from 96.3 ± 0.88% germinating at day 1 to 93.7 ± 0.88% after 3 weeks, where error is SD), we found that conidia that were impregnated on the sheets gradually lost viability (from 95.0 ± 1.0% germinating after 1 day to 82.7 ± 6.17% after 1 week, 70.7 ± 7.35% after 2 weeks, and 63.0 ± 6.7% after 3 weeks).

The proportion of fungus-infected mosquitoes observed in the study was combined with baseline data from a well-characterized nearby village in an adapted malaria transmission model (*19*) to estimate the impact of fungus-treated targets on the intensity of malaria transmission [entomological inoculation rate (EIR)] (SOM text). The model estimates show that fungus-impregnated sheets would have a significant impact on parasite transmission. Even with just 23% of the mosquitoes in houses acquiring an infection, as obtained in this experimental study, the EIR could be reduced from a baseline level of 262 infective bites per person per year to 64 (i.e., 75% reduction of transmission intensity; Fig. 2). The proportion of mosquitoes with sporozoites in the overall population would decline from 0.011 to 0.0036 (fig. S2). Relatively simple modifications such as larger sized sheets, higher conidial dosages, and improved efficacy of the conidial formulation are all expected to increase considerably the overall proportion of mosquitoes that become infected and therefore the effectiveness of the intervention. For example, increased coverage of mosquito resting sites could improve impact further such that a still modest proportion of 50% of mosquitoes becoming infected would reduce the EIR by 96%.

We conclude that the application of fungal pathogens to kill adult malaria vectors could significantly reduce parasite transmission and therefore lead to reduced malaria risk. This finding, together with the reported reduced bloodfeeding propensity of fungus-infected female mosquitoes (*12*, *20*) and possible negative effects of fungal infection on *Plasmodium* development in the mosquito (*12*), demonstrates that this method of biological control has potential as a new strategy for malaria control.

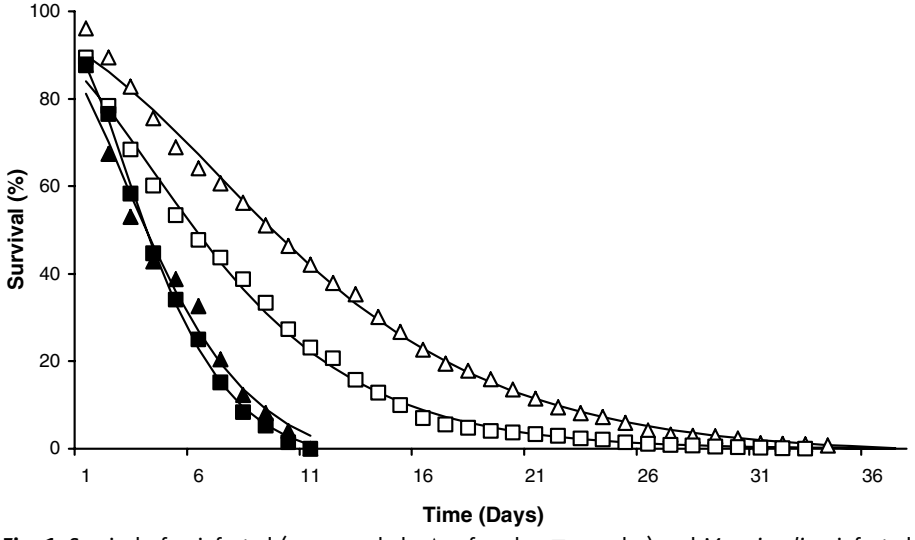

Fig. 1. Survival of uninfected (open symbols: Δ = females, □ = males) and *M. anisopliae*–infected (closed symbols: ▲ = females, ■ = males) wild *A. gambiae s.l.* mosquitoes collected from rural Tanzanian houses. Data fit to the Gompertz survival distribution model.

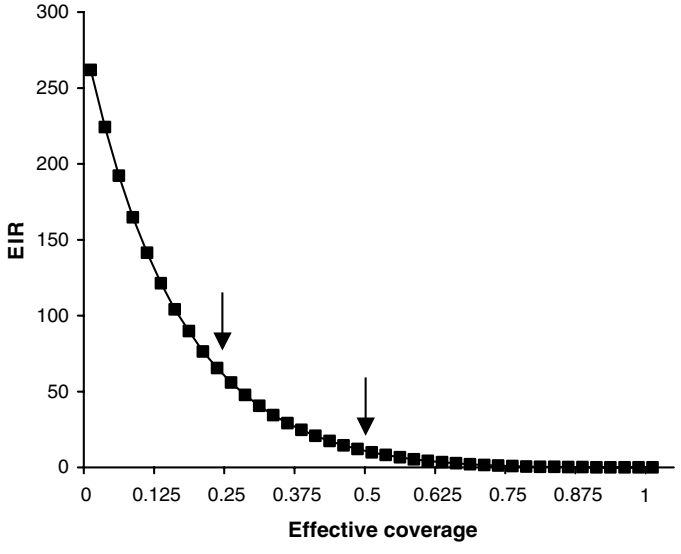

Fig. 2. Predicted relationship between effective coverage with *M. anisopliae*–treated cloths and reduction in EIR. Arrows show the EIR at coverage of 0.228 as achieved in the field trial (left arrow) and the anticipated effect of increasing it to 50% (right arrow).

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Endosomal Proteolysis of the Ebola Virus Glycoprotein Is Necessary for Infection

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Ebola virus (EboV) causes rapidly fatal hemorrhagic fever in humans and there is currently no effective treatment. We found that the infection of African green monkey kidney (Vero) cells by vesicular stomatitis viruses bearing the EboV glycoprotein (GP) requires the activity of endosomal cysteine proteases. Using selective protease inhibitors and protease-deficient cell lines, we identified an essential role for cathepsin B (CatB) and an accessory role for cathepsin L (CatL) in EboV GP-dependent entry. Biochemical studies demonstrate that CatB and CatL mediate entry by carrying out proteolysis of the EboV GP subunit GP1 and support a multistep mechanism that explains the relative contributions of these enzymes to infection. CatB and CatB/CatL inhibitors diminish the multiplication of infectious EboV-Zaire in cultured cells and may merit investigation as anti-EboV drugs.

Ebola virus (EboV) is a member of the *Filoviridae* family of enveloped viruses with nonsegmented negative-sense RNA genomes (*1*). EboV infection is initiated by the fusion between viral and host cell membranes, which is mediated by the viral membrane glycoprotein (GP) (*2*, *3*). Mature GP is a trimer of three disulfide-linked GP1-GP2 heterodimers that are generated by the proteolytic cleavage of the GP0 precursor polypeptide during virus assembly (*4*–*6*). The membrane-distal subunit, GP1, mediates viral adhesion to host cells and is proposed to regulate the transmembrane subunit GP2, which carries out membrane fusion (*7*–*9*). The processing and function of EboV GP are analogous to those of other type-I envelope glycoproteins, such as Env of the human immunodeficiency virus (HIV) and HA of the influenza virus (*4*, *7*, *10*–*12*). Current models of infection by these viruses (*11*) indicate that a specific signal within susceptible cells, such as receptor binding or exposure to acid pH, triggers the destabilization of intersubunit contacts, conformational rearrangement of the transmembrane subunits, and membrane fusion.

The triggering signal for the EboV GP1-GP2 trimer is unknown. Specifically, an essential EboV receptor analogous to CD4/CCR5 for HIV Env has not been identified (*13*). EboV infection is blocked by inhibitors of endosomal acidification (*2*, *3*), indicating that this virus uses an acid-dependent pathway to enter cells. However, acid pH does not induce GP-dependent cell membrane fusion (*8*), as might be expected from studies of acid pH–triggered influenza virus and retroviruses (*14*, *15*). These findings suggest the possibility that a critical host factor for EboV entry is dependent on acid pH. The nonenveloped mammalian reoviruses provide such a precedent: They require the activity of acid-dependent endosomal proteases to enter cells (*16*–*18*).

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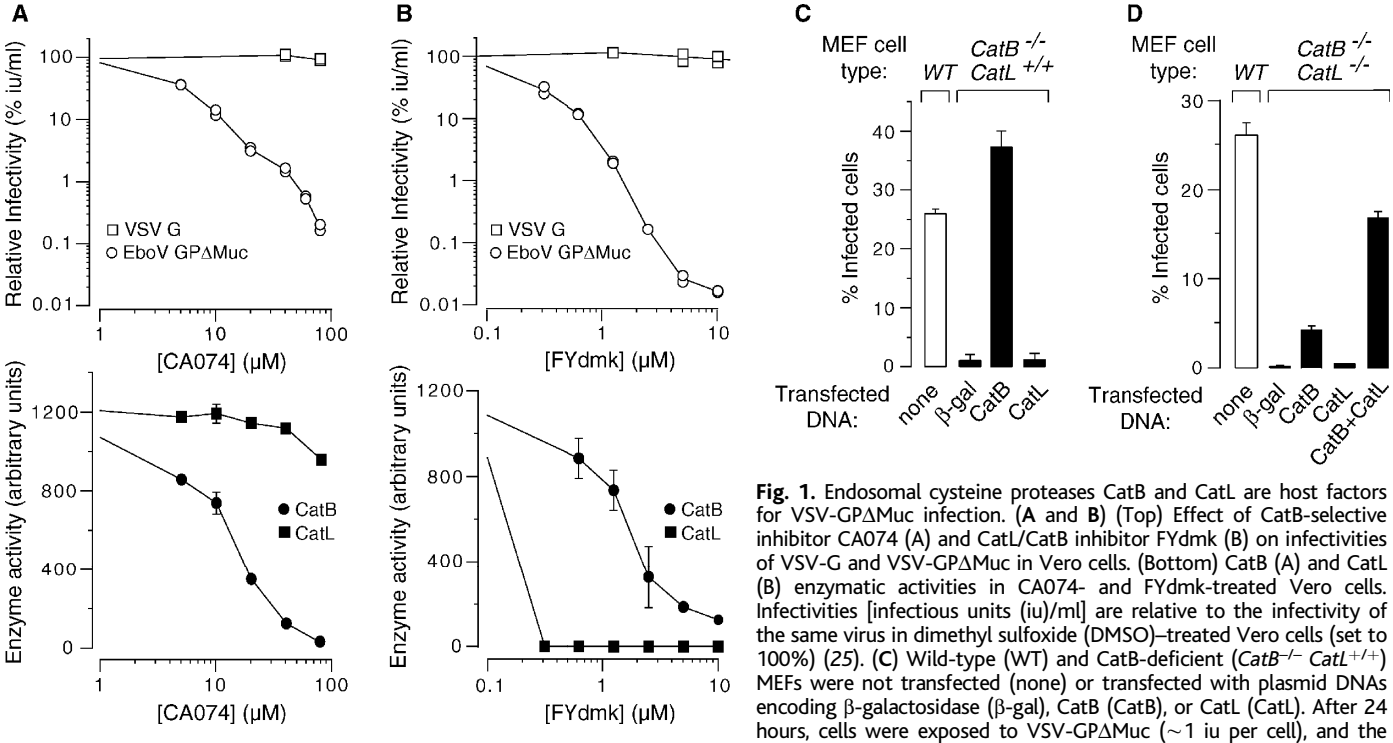

Fig. 1. Endosomal cysteine proteases CatB and CatL are host factors for VSV-GPΔMuc infection. (**A** and **B**) (Top) Effect of CatB-selective inhibitor CA074 (A) and CatL/CatB inhibitor FYdmk (B) on infectivities of VSV-G and VSV-GPΔMuc in Vero cells. (Bottom) CatB (A) and CatL (B) enzymatic activities in CA074- and FYdmk-treated Vero cells. Infectivities [infectious units (iu)/ml] are relative to the infectivity of the same virus in dimethyl sulfoxide (DMSO)–treated Vero cells (set to 100%) (*25*). (**C**) Wild-type (WT) and CatB-deficient (*CatB*$^{-/-}$ *CatL*$^{+/+}$) MEFs were not transfected (none) or transfected with plasmid DNAs encoding β-galactosidase (β-gal), CatB (CatB), or CatL (CatL). After 24 hours, cells were exposed to VSV-GPΔMuc (~1 iu per cell), and the percentage of infected cells was determined 24 hours later by flow cytometry (*25*). (**D**) Capacity of VSV-GPΔMuc to infect CatB/CatL-deficient (*CatB*$^{-/-}$ *CatL*$^{-/-}$) MEFs was determined as in (C). Infectivities from two replicates are shown in (A) and (B) and are representative of four independent experiments. Error bars, SD from at least three replicates.

To test the possibility that acid-dependent endosomal proteases are also host factors for EboV GP-dependent entry, we assessed the capacity of broad-spectrum protease inhibitors to block infection by vesicular stomatitis virus (VSV) particles pseudotyped with EboV GP (VSV-GP). The cysteine protease inhibitor E-64d specifically reduced VSV-GP infection in Vero African green monkey kidney epithelial cells by >99.9% (fig. S1). A similar profile of inhibition was observed with more highly infectious VSV particles containing a form of EboV GP that lacks the mucin-like/variable (Muc) domain in GP1 (VSV-GPΔMuc) (*6*, *19*). These findings indicate that one or more cysteine proteases in Vero cells are required for EboV GP-dependent entry.

Cathepsin B (CatB) and cathepsin L (CatL) are E-64d–sensitive cysteine proteases that are present in endosomes and lysosomes and are active at acid pH in the broad range of mammalian cells susceptible to EboV infection (*2*, *20*, *21*). To examine the roles of these enzymes, we studied the effect of a selective CatB inhibitor [CA074 (*22*)] and a CatL/CatB inhibitor [FYdmk (*23*)] on VSV-GPΔMuc infection of Vero cells. Infection was inhibited in a manner that correlated closely with the inactivation of CatB but not CatL (Fig. 1, A and B). We next measured VSV-GPΔMuc infectivity in murine embryo fibroblasts (MEFs) derived from wild-type and CatB-deficient (*CatB*$^{-/-}$ *CatL*$^{+/+}$) mice (*24*, *25*) (Fig. 1C). We observed a >90% reduction in EboV GPΔMuc-dependent infection of *CatB*$^{-/-}$ *CatL*$^{+/+}$ MEFs but no reduction in infection dependent on VSV's own glycoprotein (VSV G) (*26*). VSV-GPΔMuc infection was enhanced by the expression of CatB but not CatL. Together, these results indicate that CatB is an essential host factor for EboV GP-dependent entry.

The inactivation of both CatB and CatL with high concentrations of FYdmk (Fig. 1B) or with a combination of CA074 and FYdmk (fig. S5) inhibited VSV-GPΔMuc infection more effectively than did the inactivation of CatB alone with CA074 (Fig. 1A and table S1), suggesting a role for CatL in entry. To investigate this possibility, we measured VSV-GPΔMuc infectivity in MEFs derived from CatB/CatL-deficient (*CatB*$^{-/-}$ *CatL*$^{-/-}$) mice (Fig. 1D) (*27*). We observed a >99% reduction in EboV GPΔMuc-dependent infection of *CatB*$^{-/-}$ *CatL*$^{-/-}$ MEFs but no reduction in VSV G–dependent infection (*26*). VSV-GPΔMuc infection was enhanced by the expression of CatB but not CatL, providing additional evidence for the essential role of CatB. Although CatL is neither necessary nor sufficient for entry, we observed a synergistic increase in infection upon coexpression of CatL with CatB, suggesting that CatL enhances infection by contributing to the CatB-dependent entry mechanism.

To further investigate the roles of CatB and CatL in EboV GP-dependent entry, we

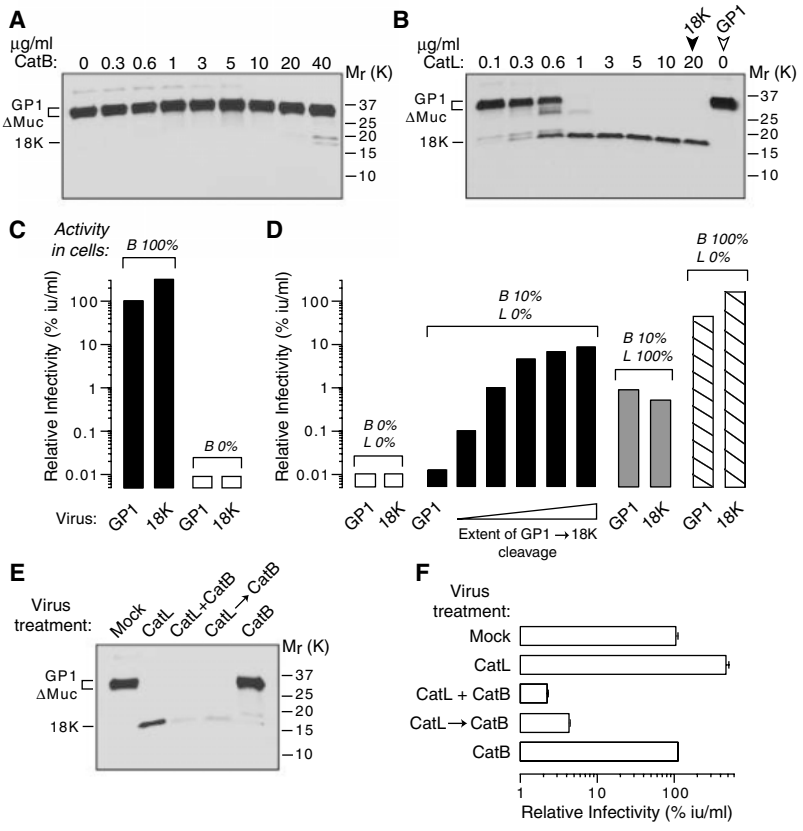

Fig. 2. Endosomal cysteine proteases act directly on EboV GPΔMuc to mediate infection of Vero cells. (**A** and **B**) Purified CatB (A) and CatL (B) cleave GPΔMuc to $GP1_{18K}$. VSV-GPΔMuc was incubated with enzyme for 1 hour at pH 5.5 and 37°C. Mock-treated VSV particles containing GP1 (open arrowhead) and CatL-treated VSV particles containing $GP1_{18K}$ (18K) (solid arrowhead) were used in (C) and (D). (**C**) VSV particles containing $GP1_{18K}$ are highly infectious and fully dependent on cellular CatB activity. Cells were not treated (solid bars) or pretreated with E-64d (300 μM) (open bars) to inactivate CatB. Approximate CatB activity (B%) in these cells is indicated above the bars. (**D**) VSV particles containing $GP1_{18K}$ bypass a block to GP1 cleavage within cells. Cells were treated with inhibitors to obtain the approximate levels of cellular CatB (B%) and CatL (L%) activity shown (also see Fig. 1 and table S1). Open bars, 300 μM E-64d; solid black bars, 10 μM FYdmk; solid gray bars, 40 μM CA074; striped bars, 1 μM FYdmk. Cells were then infected with VSV-GPΔMuc containing GP1 only, $GP1_{18K}$ only, or increasing amounts of $GP1_{18K}$ (wedge) (generated by incubation with increasing concentrations of CatL for 1 hour at pH 5.5 and 37°C). (**E**) Purified CatB efficiently digests CatL-derived $GP1_{18K}$. VSV-GPΔMuc was incubated with the indicated enzymes for 1 hour at pH 5.5 and 37°C {CatB, 40 μg/ml; CatL, 20 μg/ml; CatB and CatL together (CatB + CatL); or CatL followed by CatB [CatL→CatB (30 min each)]}. (**F**) Digestion of CatL-derived $GP1_{18K}$ by CatB inactivates VSV-GPΔMuc. Infectivities of VSV particles from (E) are shown. Averages of two replicates are shown in (C) and (D) and are representative of three independent experiments. Error bars, SD from three replicates; M_r, relative molecular weight in kilodaltons (K).

examined the effect of the purified enzymes on VSV-GPΔMuc at pH 5.5 and 37°C (Fig. 2, A and B). Both enzymes cleaved the GP1 subunit to yield an ~18-kD N-terminal fragment ($GP1_{18K}$); however, CatL mediated GP1→$GP1_{18K}$ cleavage much more efficiently than did CatB under these selected conditions. After complete GP1→$GP1_{18K}$ cleavage by CatL, VSV particles remained fully infectious and dependent on cellular CatB activity in Vero cells (Fig. 2C) and MEFs (*26*), indicating that GP1→$GP1_{18K}$ cleavage is not the step in entry that specifically requires CatB.

On the basis of these findings, we pursued the hypothesis that VSV particles containing $GP1_{18K}$ are an intermediate in the CatB-dependent entry pathway. This hypothesis predicts that the $GP1_{18K}$-containing particles should bypass a block to infection of cells in which presumptive GP1 cleavage by cellular CatB and/or CatL is inhibited. Accordingly, we treated cells with inhibitor to reduce CatB activity to ~10% and CatL activity to undetectable levels, and we then challenged those cells with CatL-treated VSV particles containing increasing amounts of $GP1_{18K}$ (Fig. 2D). Infection of these cells was enhanced in a $GP1_{18K}$-dependent manner, indicating that GP1 cleavage is an essential step in entry. In contrast, we observed little or no $GP1_{18K}$-dependent enhancement of infection in cells with ~100% CatL or ~100% CatB, indicating that the required intracellular cleavage of GP1 that is mimicked by in vitro

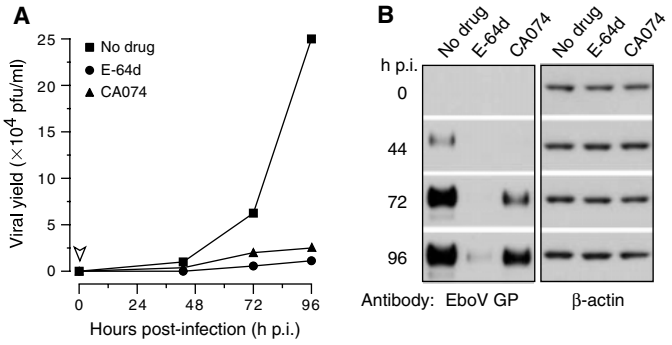

Fig. 3. Endosomal cysteine protease inhibitors diminish EboV-Zaire multiplication in Vero cells. (**A**) Yields of infectious EboV-Zaire released from cells treated with DMSO (no drug), 300 μM E-64d (to inactivate endosomal cysteine proteases), or 80 μM CA074 (to selectively inactivate CatB) for 4 hours are shown. Growth medium containing inhibitors was removed from cells at the indicated time (arrowhead) and replaced with fresh medium lacking inhibitors. pfu/ml, plaque-forming units per milliliter; h p.i., hours post infection. Averages from two replicates are shown. (**B**) GP expression in EboV-infected cells from (A). β-actin was used as a loading control.

GP1→$GP1_{18K}$ cleavage is mediated by CatL and/or CatB.

VSV particles containing $GP1_{18K}$ remain dependent on cellular CatB (Fig. 2, C and D), indicating the existence of a downstream CatB-dependent step. Purified CatB but not CatL efficiently digested CatL-derived $GP1_{18K}$ to fragments not detected by immunoblot (Fig. 2E) and inactivated infectivity by >90% (Fig. 2F). More work is needed to uncover the mechanistic details of this CatB-dependent step, but our results are consistent with a simple model in which CatB-mediated digestion of $GP1_{18K}$ in vitro inactivates VSV particles by relieving GP1-dependent constraints on GP2 and inducing premature deployment of the fusion machinery. In this scheme, EboV GP1 digestion by cellular CatB and CatL is functionally equivalent to fusion-triggering signals for other viruses, such as the binding of retroviruses to receptors (*15*) and the exposure of influenza virus to acidic pH (*14*, *25*).

Taken together, our findings indicate that GP1 proteolysis by CatB and CatL during entry is a multistep process. We propose that this process is initiated by cleavages of GP1 by CatB and/or CatL to remove C-terminal sequences (figs. S3 and S4) (*25*) and to generate an N-terminal $GP1_{18K}$-like species, which is then digested by CatB to trigger membrane fusion and entry. Our data suggest that CatL contributes to infection, particularly when CatB activity is low, by virtue of its ability to mediate initial GP1 cleavages, but is insufficient for entry because further digestion of GP1 requires CatB. The C-terminal region of GP1 contains highly variable and heavily glycosylated sequences, including the Muc domain (*6*), which promote viral adhesion (*9*) and may shield viral particles from immune recognition, but may have to be removed first to allow further GP1 digestion. An analogous multistep strategy is used by HIV: CD4-receptor binding is required for Env to be triggered by the CCR5 coreceptor (*11*). Even more striking parallels exist between the enveloped Ebola virus and the nonenveloped mammalian reovirus: reovirus entry also requires stepwise proteolysis of viral surface proteins by endosomal cysteine proteases (*16*–*18*).

Human fatalities from EboV infection range from 50 to 90% and treatment is currently restricted to supportive care (*1*). The development of an antiviral therapy for EboV is therefore a high priority. To examine whether endosomal cysteine proteases are potential anti-EboV targets, we measured the effects of E-64d and CA074 on growth of the Zaire strain of Ebola virus. Vero cells were pretreated with these inhibitors and exposed to virus for 1 hour. Inhibitor and unbound virus were then removed and viral growth was monitored. The yields of infectious EboV progeny (Fig. 3A) and expression of cell-associated GP1 (Fig. 3B) were markedly reduced in inhibitor-treated cells, suggesting that EboV multiplication in Vero cells is sensitive to inhibitors of endosomal cysteine proteases in general and of CatB in particular. Further investigation of the antiviral efficacy of such inhibitors may therefore be warranted. The wealth of existing knowledge regarding the design (*21*) and in vivo pharmacology (*28*) of these inhibitors may facilitate development of an anti-EboV therapy.

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29. We thank J-Y. Yang, V. Spivak, and P. Popernack for technical support; C. Peters and AstraZeneca for knockout mice; T. S. Dermody and N. Fehrenbacher for protease plasmids and MEFs; and M. A. Agosto, S. C. Harrison, T. W. Geisbert, T. Ivanovic, P. B. Jahrling, J. Jané-Valbuena, M. Lazar, W. Mothes, G. J. Nabel, M. L. Nibert, T. Raz, and S. Viswanathan for helpful discussions. Supported by grants from NIH (J.M.C., N.J.S., and S.P.W.), the Cystic Fibrosis Foundation (S.P.W), and departmental funds (S.P.W.). K.C. is supported by the New England Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research. U.F. is supported by an Emmy Noether grant from the Deutsche Forschungsgemeinschaft.

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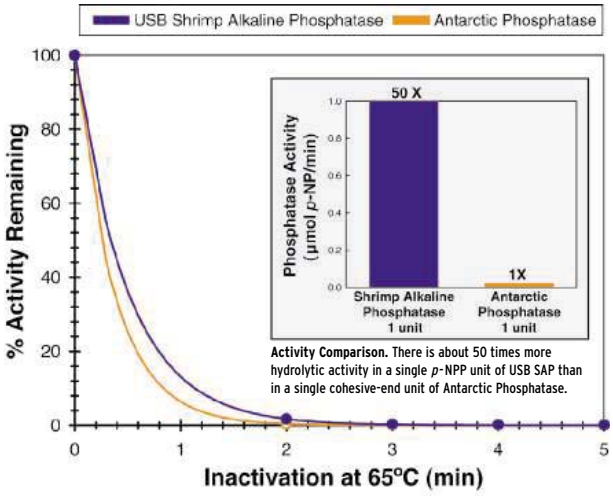

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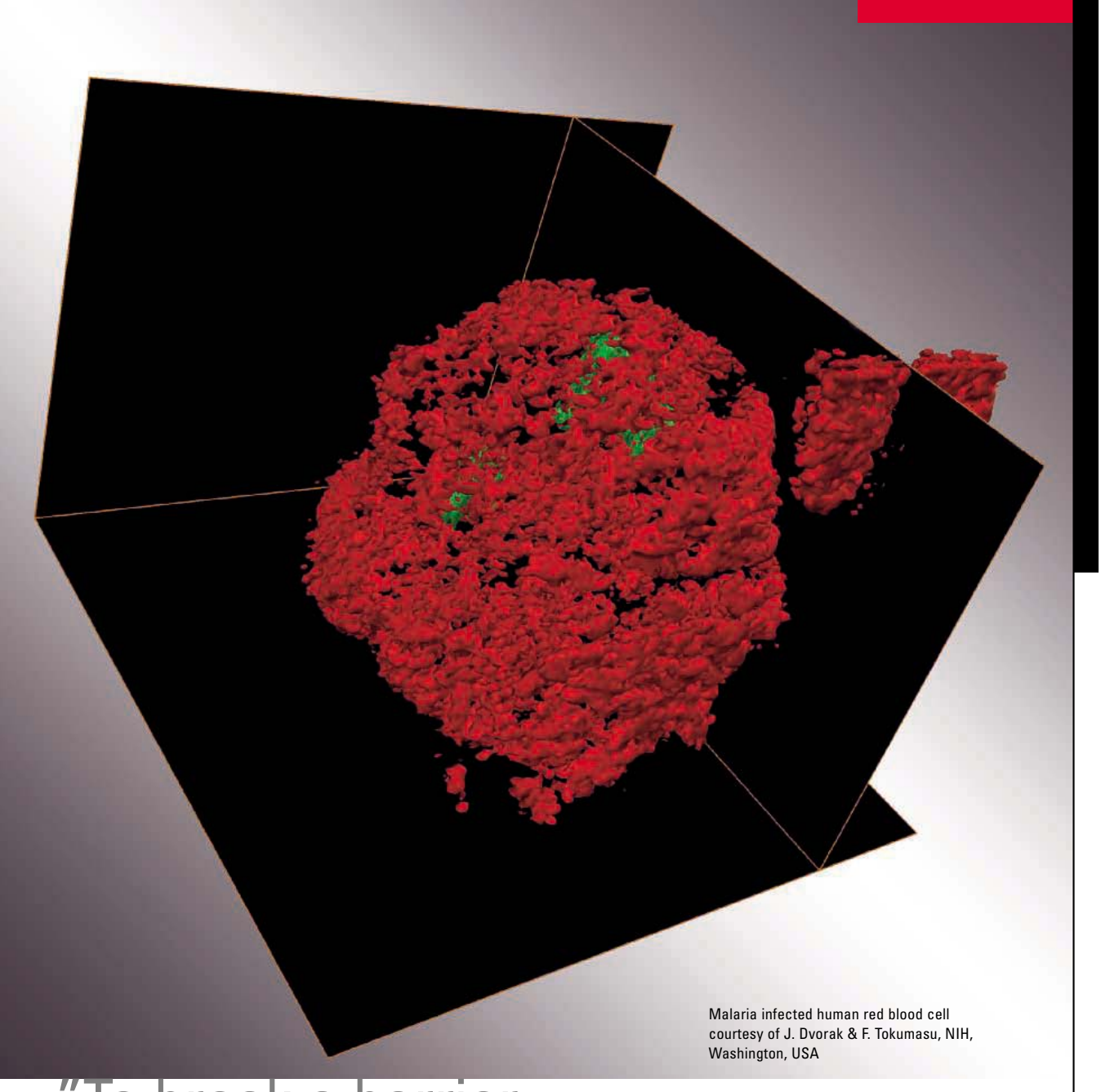

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In this issue:

- DNA isolation and purification
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- Laboratory automation and liquid handling
- Bioinformatics
- Hardware for sequencing

The successful sequencing of the human genome five years ago started an all-out effort to apply the results to improve understanding of the bases of life and to sequence the genomes of a variety of other organisms. "Having the human genome sequence has enabled a whole new approach to biological research," says Noreen Galvin, GenomeLab business manager at **Beckman Coulter**. "Scientists can now look at questions in a more significant way." To support those efforts, scientists and vendors have set out to upgrade methods of sequencing DNA.

Steady, Not Startling

The follow-up efforts have relied on steady improvements rather than startling advances in approaches to sequencing. "Key tools that are currently used are basically the same as a few years ago, but sequencing has become more robust and user friendly," says Jürgen Lauber, head of sequencing services at **Qiagen**. "Innovations have been missing but, we think, not necessary," adds Thomas Uschkureit, product line manager for real-time PCR instruments at **Eppendorf**. "Vendors have introduced improvements in handling, smaller capillaries, better resolution, and some reagents." Steven Scherer, head of mapping in the Human Genome Sequencing Center at the **Baylor College of Medicine**, agrees. "There has been more building on the success and focusing on fresh organisms for comparative genomics," he says. "We have seen some nibbling around the edges in technology development like sequencing by synthesis and by hybridization, the lab-on-a-chip, workflow optimization on **Applied Biosystems** machines, development of machines using multiple lasers, and massively parallel short sequence reads."

New tools respond in large part to new demands. "We are still interested in sequencing genomes of other organisms, but we are focusing more on medical sequencing [often called resequencing]," Scherer continues. "There's also the idea of sequencing more humans to give us a better handle on human variation. A center like ours will begin to use the human genome to establish the link between genes' variations and disease phenotypes." Michele Paris, director of marketing for **USB Corporation**, pinpoints another research target. "The need to look at short sequences is increasing," she says.

Cost saving and user friendliness have also emerged as key themes. "A lot of scientists are aiming for cost efficiency," says Kareem Saad, worldwide executive in clinical genomics at **IBM Healthcare and Life Sciences**. "We firmly believe that, not too long from now, gene expression MORE ›››

Inclusion of companies in this article does not indicate endorsement by either AAAS or Science*, nor is it meant to imply that their products or services are superior to those of other companies.*

This is the second of four special supplements this year on Advances in Genomics. The first appeared in the 11 February issue of Science*; the next will appear in the 29 July issue.*

» advances in: Genomics

experiments will become routine for individuals." In addition, the emergence of high throughput sequencing has put a spotlight on data management. "Another area of importance is informatics," says Lauren Seilnacht, senior manager of the product line for genetic analysis at Applied Biosystems. "There have been many improvements in software and base calling."

Isolation, Purification, and PCR

Any sequencing project must begin with pure materials. "When sequencing started, it was essential to have ultrapure DNAs," says Qiagen's Lauber. "Although sequencing chemistry has become more robust, isolation and purification of DNA is still an issue. Since core facilities that do research for smaller organizations must have success every time, they must focus on isolation and purification." **GE Healthcare** and **Roche Applied Science** have developed proprietary resins for isolating DNA. And such vendors as **BD Biosciences Clontech**, **Promega**, and **Stratagene** offer reagents and kits for DNA isolation and purification.

Qiagen has more than 300 relevant products on the market. "Even if you don't have enough starting material, you can use our new whole genome amplification technology," Lauber says.

In addition, a new generation of automated equipment, some of it based on microfluidic technology, can perform all of the steps required for isolating DNA. **Agilent Technologies** provides an example with its 2100 Bioanalyzer for sample preparation and other procedures that involve DNA.

Colony PCR is an alternative method to produce sequencing templates. "It's a very important tool as the DNA template's purity is critical and PCR is a significant method of producing that," says Tuomas Tenkanen, R&D director of Finnish firm **Finnzymes**. "Upfront reactions have to be done using PCR," adds Andy Felton, product manager for real-time PCR instruments at Applied Biosystems. Felton's firm has developed its AmpliTaq Gold DNA Polymerase, a chemically modified form of DNA polymerase that releases the active AmpliTaq enzyme at the appropriate time and temperature.

Finnzymes, meanwhile, has developed what it calls the Phusion DNA Polymerase. This generates templates with an accuracy and speed previously unattainable with a single enzyme, even with the most difficult templates. "We have combined all the necessary features in one single enzyme," Tenkanen says. "We have achieved this by using a proofreading polymerase that is fused with a double-stranded DNA binding protein."

Achievement Awards at Gala Dinner

The presentation ceremony for the Fourth Annual Pharmaceutical Achievement Awards will be held on the evening of Monday, August 8 at Boston's State Room. The 17 awards honor significant scientific, business, and philanthropic accomplishments in the global pharmaceutical and biotechnology industries. A diverse, 35-person executive board determines the finalists and winners. You can obtain a complete listing of the 2005 finalists and details on how to participate in the awards dinner from the website:

» http://www.pharmawards.com

Tenth Anniversary Congress

The Boston Convention & Exhibition Center will host the10th anniversary Drug Discovery Technology World Congress between August 8 and August 12 this year. The event, sponsored by **IBC Life Sciences**, is intended to provide advice to attendees on ways to accelerate the drug discovery and development process, to expand the clinical pipelines of promising drugs and biologics, and to reduce attrition of compounds owing to problems involving safety and efficacy.

Speakers will present case studies and strategies of real drug discovery research projects in three scientific streams: promising approaches to drug discovery and drug development; success factors in the transition from discovery to clinic; and the development of protein and antibody therapeutics. Speakers will also illustrate how technologies impact their discovery efforts and productivity. Other presentations will focus on business ventures and deal-making, gaining value from discovery information, and accessing Asia Pacific business opportunities and collaborations. The event will also feature a showcase of emerging and early stage companies. And about 200 companies will participate in the exhibition hall on the middle three days of the event. More information is available at the website

» http://www.drugdisc.com

Sequencing Systems

The discovery of restriction enzymes and DNA polymerases made the sequencing of DNA possible. Companies such as **New England Biolabs**, Promega, and USB soon saw the value of commercializing those bioreagents. They produced them first as stand-alone reagents and then as kits that enable researchers who lack training in molecular biology to work in the field. Despite the development of more advanced sequencing techniques, USB's Paris says, "Restriction enzymes are not going away any time soon. We have seen the return of some researchers into the manual sequencing arena, looking to do quick analyses of short DNA sequences without any sophisticated instrumentation."

For more ambitious projects, however, scientists turn to automated DNA sequencers that perform gel electrophoresis, scan gels, and determine the sequences of nucleotide bases without constant attention from laboratory researchers and technicians. Companies that have developed these instruments include Beckman Coulter, GE Healthcare, and **LI-COR**. Applied Biosystems has a family of DNA analyzers that sequence and genotype genes. They range from the single capillary 310 system to the 96-capillary 3730 XL. "These heavy-hitting instruments will help to sequence the next 12 de novo genomes, including the marmoset, sea slug, and various fungi," Seilnacht says.

Elsewhere, the Genome Sequencing System recently introduced by **454 Life Sciences** provides high throughput sequencing with a footprint no larger than a desktop. According to the company, it enables a single scientist to prepare and sequence an entire genome of any size after performing a single sample preparation. MORE ›››

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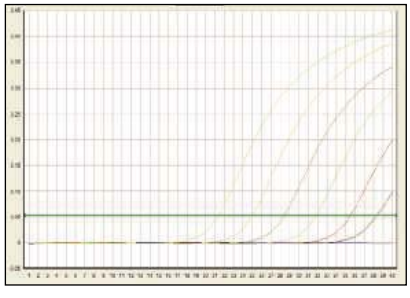

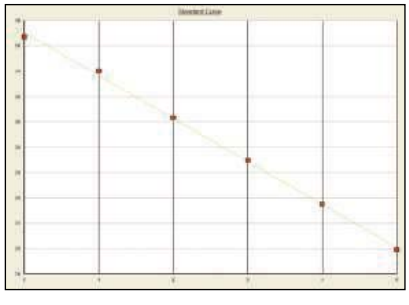

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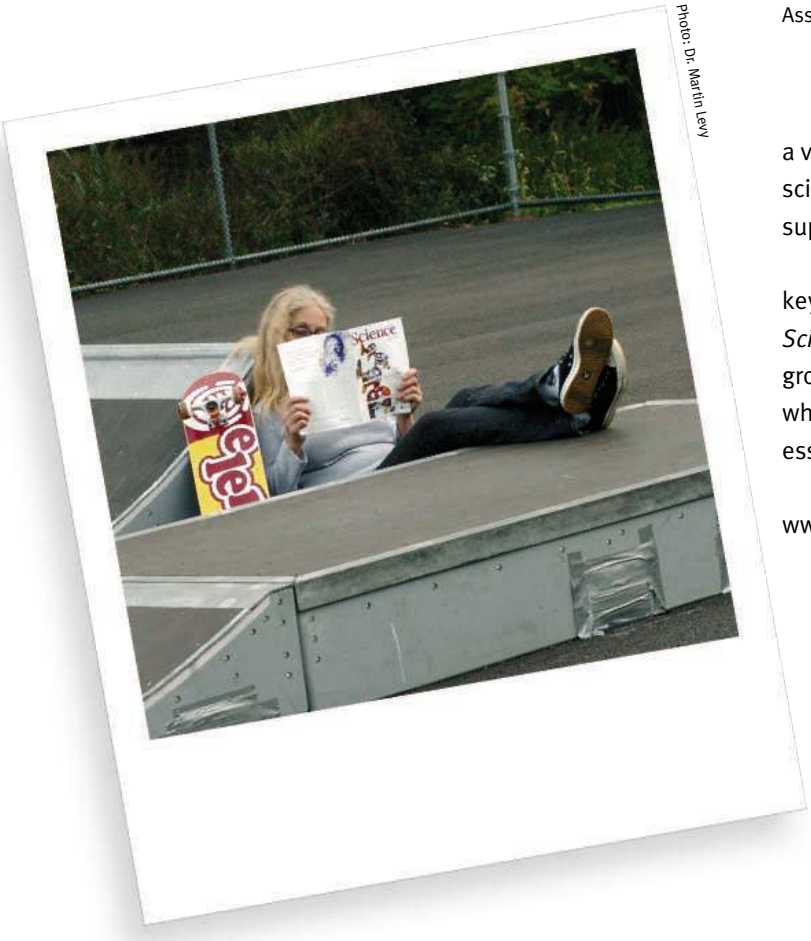

Photo: Dr. Martin Levy

Lorraine Kuhn at Ardsley Village Skatepark

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» advances in: Genomics

Automation and Robotics

Automation and robotics have also played a key role in DNA sequencing, by relieving researchers of the need to perform repetitive operations on large numbers of samples. "Automated workstations are essential for preparation," says Eppendorf's Uschkureit. "From the start of genome sequencing, one of the biggest things about them was their reliability – the removal of human error and the quantitation of DNA," adds Beckman Coulter's Galvin. Instruments from companies such as **Bio-Tek Instruments**, **Hamilton Company**, and PerkinElmer decrease the time that scientists require to complete their experiments since they can be programmed to run during off hours in the absence of humans.

Eppendorf has introduced what it calls the epMotion family of products. The modular family offers flexible expansion options for a broad spectrum of applications, enabling life scientists to carry out a wide variety of dispensing and contamination-free pipetting tasks in the laboratory. "We call them smart workstations," Uschkureit says. "They have a high precision of less than 5 percent in one microliter pipetting. They also have small footprints."

Beckman Coulter offers a complete range of automation solutions for every throughput demand in research, from modular liquid handling systems to integrated robotic systems. Its Biomek 3000 Laboratory Automation Workstation provides researchers with simple, intelligent automation of liquid handling tasks from pipetting to diluting and dispensing operations, plus a wide range of validated automated chemistries that include PCR setup, reaction cleanup, sample quantitation and normalization, and DNA amplification and hybridization. "It's an open and flexible platform like a robot," Galvin says. "The system has simplicity and scalability." Other suppliers, among them **Caliper**, Eppendorf, and **Genomic Solutions**, have created robotic systems that perform many of the upstream and downstream processing tasks necessary for DNA sequencing.

More Data in Less Time

While improved tools for DNA sequencing permit researchers to produce more data in less time, they also create immense amounts of genomic information – amounts that are impossible to track and manage with traditional laboratory methods. Computer storage of sequencing data can require hard drives with hundreds of gigabytes of capacity.

Bioinformatic programs play critical roles in interpreting those vast volumes of sequence data. Thus, bioinformatics providers such as **Accelrys**, **Invitrogen**, **Nucleics**, and **Textco** offer suites of programs for work with DNA sequences.

In addition, information technology companies **Apple**, **Hewlett-Packard**, IBM Life Sciences, and **Sun Microsystems** are creating systems that will allow life scientists to perform their daily tasks without interruption. "We're in the business of developing the underlying technologies, foundations, and architecture, along with implementation methods and the necessary hardware," IBM's Saad says. "We have an entire portfolio of solutions and architectures. They are relevant to sequencing in that all the information needs to be linked back to the specific gene of interest."

Plainly, scientists who perform DNA sequencing aren't resting on the laurels they won by elucidating the human genome. Backed by improved tools, they continue to sequence more genomes each year, helping to expand basic knowledge of genes, their sequences, and their relationships to each other.

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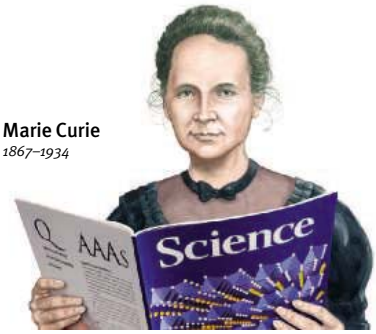

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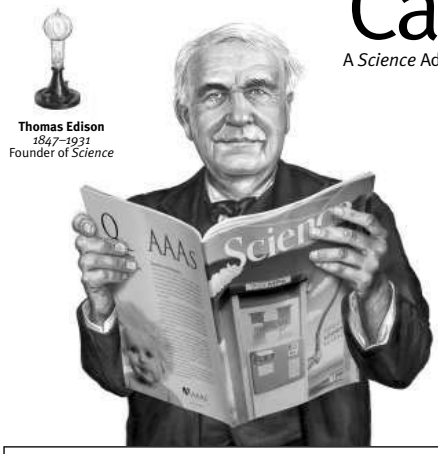

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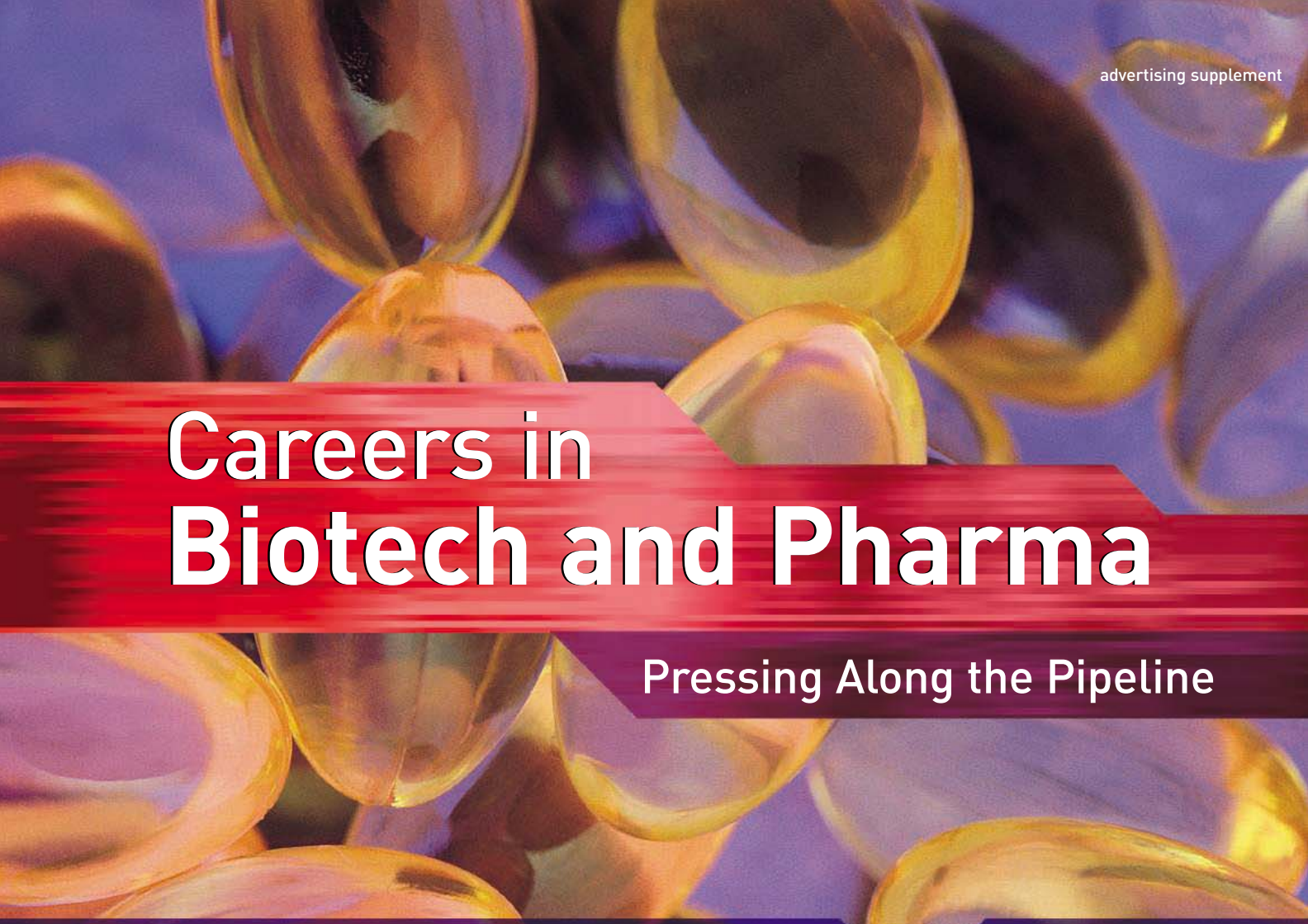

Careers in Biotech and Pharma

Pressing Along the Pipeline

TO MAINTAIN THEIR DRUG PIPELINES, BIOPHARMACEUTICAL COMPANIES PAY GROWING ATTENTION TO THE ACTIVITIES THAT FOLLOW THE DISCOVERY OF PROMISING MOLECULES. THAT WORK DEMANDS SCIENTIFIC SKILLS AND MINDSETS DIFFERENT FROM THOSE ASSOCIATED WITH DISCOVERY. BY PETER GWYNNE

For the past decade, biopharmaceutical firms have created more promise than products. They have spent increasing amounts on research but have received little benefit from those expenditures in terms of approved drugs. "Everyone in the pharmaceutical industry is aware that things have fallen off in terms of the number of products in the pipeline reaching regulatory agencies for approval," comments Barry Gertz, executive vice president of clinical quantitative sciences at Merck. That doesn't mean that drug discovery teams have failed in their mission to identify molecules with therapeutic potential. Rather, says Peter Smith, senior vice president, drug safety and disposition at Millennium Pharmaceuticals, "It has to do with the interface between discovery and development; discovery people would throw their molecules over the wall for development."

No more. Conscious of their bottom lines, pharmaceutical corporations and biotechnology firms in the drug development business have started to emphasize both the handoff from the discovery to the development segments of the drug pipeline and the need to spend more in terms of money and manpower on postdiscovery work. "Development is becoming a major focus for all companies, to get return on their large expenditure on discovery," says Jack Dean, head of U.S. research and development for sanofi-aventis. "There's quite a bit of pressure for companies – and particularly young ones – to move their resources to postdiscovery," agrees Kleanthis G. Xanthopoulos, CEO of Anadys Pharmaceuticals.

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Research Scientist, Pulmonary Diseases

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We are looking for a biologist who has a good understanding of the literature and working knowledge of the pathological and immunological mechanisms associated with pulmonary diseases. The candidate will be expected to work independently to establish in vitro and in vivo models of lung pathology by applying state-of-the-art knowledge of scientific principles. Candidate will also lead research efforts to identify new disease indications in support of development projects. Candidate should have a BS with at least 10 years relevant experience, MS with at least over 8 years relevant experience or PhD with 0-2 years experience.

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The position involves playing a leadership role within Oncology research and also supporting the administrative management and the development of strategic direction for the group. The candidate will be responsible for managing the identification and characterization of antibody-based and other biologic cancer therapeutics. Candidate should have a PhD, at least 8 years experience in the pharmaceutical or biotechnology industry, experience with animal models for cancer drug evaluation, experience supervising PhD level scientists, and a demonstrated record of accomplishment in oncology research.

Research Scientist, Cardiovascular

(Req. 0502760)

We are seeking a highly motivated scientist to join an established Cardiovascular/ Metabolic Diseases group responsible for preclinical research programs. The successful candidate would have excellent understanding of the mechanisms involved in the development of metabolic diseases (obesity, type 2 diabetes and metabolic syndrome), and should have hands-on experience in in vitro and in vivo models for metabolic diseases. This individual would be expected to initiate and lead efforts in new target identification and validation and provide scientific and technical support for ongoing metabolic diseases projects. Experience in drug development would be desirable.

A PhD in a related Life Science and 0-2 years bench experience in the metabolic disease area is required.

Senior/Principal Scientist, Oncology Research

(Req. 0500597)

The successful candidate will propose, design, and execute scientific research projects related to the development of biologic cancer therapies. The position requires the ability to design, conduct, and supervise the conduct of in vitro and in vivo studies testing the efficacy and safety of novel anti-cancer biologics. The successful candidate will have a PhD and/or MD and at least 5 years work experience in tumor biology, cell biology, molecular biology or a related field. 3-5 years experience in the pharmaceutical or biotechnology industries is preferred. Experience supervising PhD level scientists and a demonstrated record of accomplishment in oncology biologics research is also required.

Research Scientist, Infectious Diseases

(Req. 0502553, 0412758, 0502562)

We are seeking two BS/MS Research Scientists with expertise in immunology or microbial pathogenesis. Candidates will be responsible for performing molecular, immunologic and cell-based assays to evaluate bioactivity and dissect the mechanism of action for anti-infective biopharmaceuticals. Experience with ex vivo tissue sample analysis, RNA isolation, ELISA and reporter assays required (0502553, 0412758). We are also seeking a BS/MS Research Scientist with at least three years laboratory expertise in microbial genetics of Pseudomonas aeruginosa and other Gram-negative bacterial pathogens to establish microbial culture systems, perform site directed mutagenesis as well as immunologic and biochemical assays for evaluation of novel biopharmaceutical inhibitors of respiratory pathogens (0502562).

Research Scientists, Immunobiology

(Req. 0502448)

We are seeking a highly motivated scientist to join the Immunobiology group to support the early stage research targets for biology research related to neurodegenerative diseases. The successful candidate will have experience in standard cell culture methods as well as primary neuronal cell culture, cell-based assays, and in vitro assays (such as ELISA, Luminex) and tissue homogenization. Experience with fluorescence microscopy and imaging analysis is also desired, but not necessary. Successful candidates will have a BS/MS in biochemistry, neuroscience or related life science with 2-5 years experience, preferably in industry.

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Careers in Biotech and Pharma


JACK DEAN

New Urgency

The new urgency reflects the desire of biopharmaceutical companies to improve their efficiency in two ways: by rejecting molecules likely to fail as early as possible, and by moving the probable successes along the pipeline with all deliberate speed. "Once you take a molecule into development, you want a high likelihood that it will succeed," Smith explains. "So you assess the characteristics of druggability earlier and earlier; you have to make quick kills." And for the molecules that survive those early cuts, adds Martin Griot, head of global pharmaceutical operations at Novartis International, "The lead time is very important. We need to be on time to launch our new pipeline products."

Darryl Patrick, vice president of nonclinical development at Vertex Pharmaceuticals, outlines the basic differences between discovery and development. "Drug discovery is the process of trial and error to identify new biological targets, chemical leads, and drug candidate optimization driven by scientific curiosity," he says. "Drug development is different in that the focus is on one compound moving through a series of stages. Discovery can move in numerous scientific directions while development must move in a structured, staged process under strict regulations." In the process, he adds, drug developers can face several types of roadblock: "The challenges include inappropriate pharmacokinetic properties, toxicologic findings, nonscalable drug syntheses, and the inability to develop a pharmaceutically acceptable formulation."

Postdiscovery work has a simple goal. "Development scientists play an important role in bringing new medicines to patients around the world," says Liz Power, spokesperson for Pfizer. Achieving that goal demands teams of scientists different from those who work on discovery research. "Really it's applied research versus basic research," Smith notes. Not only do recruiters for development work seek individuals trained in fields other than the basic molecular biology and biochemistry typical of discovery scientists; they also aim to recruit scientists with a more businesslike mindset than their research colleagues.


BARRY GERTZ

A Call for Commercial Ability

"The principal difference has to do with training for regulatory, pharmacology/toxicology, and clinical development," explains Genencor International's senior vice president for health care, Mark A. Goldsmith. "Individuals who come up through the laboratory side don't typically receive the training in moving product from discovery to the marketplace. Companies have to hire people with product-focused experience obtained elsewhere or individuals who look as if they have the capabilities to be trained in-house." Griot echoes that point. "Individuals have to have commercial ability," he says. "We have training courses to help develop those people. But if they bring that in, so much the better." Just as important, postdiscovery scientists must prove themselves to be effective team players. "It is extraordinarily important to be able to work on teams," says Power. "Also, development scientists must be good problem solvers and must have strong analytical skills."

The postdiscovery segments of the drug pipeline include a wide range of activities. "You need to get your molecules through all the development hoops – registrations, preclinical research, clinical trials, technology transfer for manufacturing, and regulatory affairs," Dean points out. "The people we're looking for have training in process chemistry, chemical scale-up, analytical sciences, toxicology, drug metabolism and pharmacokinetics, pharmaceutical sciences and formulation technology, and manufacturing and packaging technology. And in the clinic we look for people with medical training who can run clinical trials, biostatisticians, nurses, technical people who can help conduct the trials, and finally people who understand the regulatory guidelines to assemble the dossiers for filing. It's a very broad team approach with multidisciplinary people involved."

Smith identifies the specific fields of training most sought by recruiters. "Toxicology, pharmacology, and drug metabolism are involved in putting together regulatory documents to get compounds into clinical trials and to apply for new drug applications," he says. "But these disciplines are used earlier and earlier – even in discovery – to test druggability, toxicity, severe organ injury, and the length of molecules' half lives."


MARK A. GOLDSMITH

Particularly Prized Scientists

Recruiters particularly prize scientists capable of linking drug discovery and development. "There's real value in people with M.D. and Ph.D. training who can work effectively with their discovery colleagues," Gertz says. "Another important group consists of physicians who are experts in particular therapeutic areas, via specialty training in residencies and fellowships."

Recruiters see relatively few scientists with the necessary portfolio of qualifications for development work. "It's a resounding issue that we don't have enough quality people out there," Xanthopoulos concedes. A major reason concerns the lack of academic training focused on drug development. "Universities have moved into molecules rather than the practical side," Dean explains. "Scientists have not been introduced to information about the drug development process. Toxicologists have all their training in molecular biology and not whole animal pharmacology." Smith agrees. "The emphasis on molecular biology has led to a movement away from some of the applied sciences necessary for drug development, such as toxicology and whole animal pharmacology," he says. "We still need animal models of disease. And drug metabolism scientists are spending more of their time on pharmacogenomic approaches. So I worry that we'll end with a shortage of the people required to put together the reports and documents that regulatory agencies need to move drugs through the pipeline."

The situation has started to improve. "Some universities are recognizing the ever-changing needs of our industry and in response continue to align their curricula to reflect the market's demands," says Ronald Schmidt, vice president of public relations at Centocor. "Students graduating from specific biotechnology programs are better prepared for our business. Universities are providing a great foundation upon which graduates can build their careers and serve as a key source for talent acquisi-

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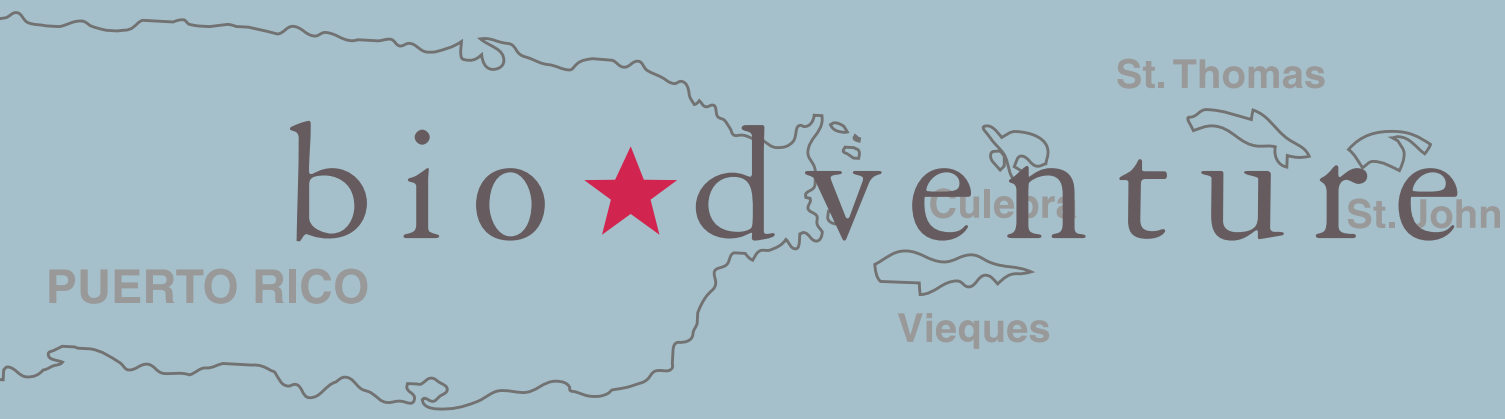

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Careers in Biotech and Pharma

tion in the scientific fields." Goldsmith takes an optimistic view of universities' plans for training would-be drug developers. "There have been courses on quantitative skills such as statistical analysis," he says. "There could be increased recognition in universities, graduate schools, and medical schools of the need to train individuals who will go into the pharmaceutical sector."


MARTIN GRIOT

Business Backgrounds

Nevertheless, recruiters often aim to attract development scientists with some background in the business. "European and other universities produce excellent life science graduates suitable for a career in drug development," says Terry Gallagher, global head of development staffing for Novartis International. "However, a large majority of our new associates are not joining directly from the university. Most of them have specialized in one of the areas important for late stage development, such as clinical trials, specific statistical methods, or drug registration. Many also have previous experience in the pharmaceutical industry."

Rich Tillyer, head of Merck's preclinical development group, has much the same experience. "It's very hard to find people from the academic world," he points out. "We get a lot of people from industry." Even when they recruit suitable scientists in academe, biopharmaceutical firms often seek individuals with industry experience to work alongside them. "Companies often look for complementing skill sets that will form the most effective drug development teams," Vertex Pharmaceuticals' Patrick points out. "The scientists who fill these roles come from many different aspects of the industry."

As an alternative to raiding other companies' scientists, some biopharmaceutical firms fill their development positions from the inside. "It is common practice for departments at Centocor in the late stages of drug development to attract internal talent from preclinical areas," Schmidt explains. "We encourage the continued development of the traditional laboratory scientists who are interested in pursuing opportunities outside discovery. With on-the-job training, these scientists can learn the skills needed to work outside the traditional lab environment."

Internal training plays a critical role in developing postdiscovery scientists. "If our candidates have the overall attributes we look for, we'll provide them with the education, guidance, and mentoring necessary to maximize their performance," Anadys Pharmaceuticals' Xanthopoulos says. "In particular we're reeducating some early discovery scientists who have the skills and desire to work down the pipeline. We're seeing a trend for people to find interest in the downstream work."

Pfizer tries to recruit locally as well as internally. "In the communities where we have R&D sites, we will often meet with local universities and colleges and talk with them about the 'talent profile' of our employees," Power explains. "We also provide job shadowing and research grants."


DARRYL PATRICK

Specific Needs and Hiring Philosophies

Biopharmaceutical firms' specific needs for development scientists depend on several factors, including the size of the companies, the state of their pipelines, and the existing balance among disciplines within their development departments. Hiring philosophies show up in the extent of training required of recruits, as well as the fields of training. "Pfizer," says Power, "is currently recruiting for positions that range from those that require a B.S. degree to those that require an M.D. or Ph.D."

Merck also recruits scientists with Bachelor's and Master's degrees for some areas of its development work. However, Tillyer explains, "We look for a lot of Ph.D.s and postdocs in chemistry. Recently we have started to hire more Ph.D.s; we're trying to get these people into the company as soon as they are ready and do not insist on postdoctoral experience." And to handle the handoff from discovery to clinical work, his colleague Gertz adds, "Our preference is to get M.D.-Ph.D.s. They can contribute to the remaining preclinical work and apply it to the early part of postdiscovery. As we have expanded, we have also provided opportunities for Ph.D.s to be involved in the later stages of drug development, such as monitoring clinical studies and writing protocols. Specifically, for Ph.D.s it is an advantage to have academic or industry experience in clinical trials."


LIZ POWER

Vertex often recruits new scientists with higher degrees and some industry experience. "We are recruiting a wide variety of scientists with varying levels of experience, and especially those with advanced degrees," Patrick says. "Experience, both academically and in the industry, truly makes a potential scientist stand out in recruiting."

Novartis hires largely at the Ph.D. and M.D. levels. "We recruit scientists from many areas, ranging from chemistry to biological and medical sciences," Gallagher says. "A combination of life sciences with information technology or a business degree can also be very interesting to us." As a global company, the firm seeks its scientists worldwide. "In Europe as well as in other parts of the world, there are a number of universities and centers that specialize in a certain area that is critical for drug development," Gallagher continues. "We try to identify these centers worldwide in order to specifically attract Ph.D.s, M.D.s, and other graduates to our open positions. We always aim to recruit the best person for the job irrespective of where a candidate is coming from. We also fully acknowledge the benefits of a diverse workforce, and therefore welcome applications form different backgrounds."

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pharmaceutical research

MERCK

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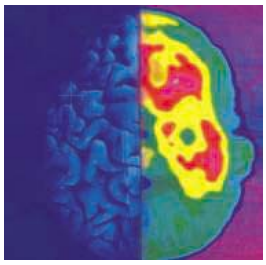

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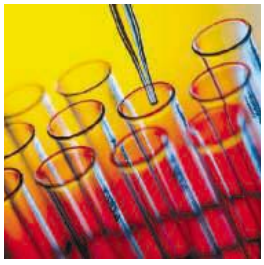

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Careers in Biotech and Pharma


PETER SMITH

From Pharmacologists to Informaticists

Another pharma based in Europe, sanofi-aventis, has a broad list of needs for work along its drug pipeline. "We recruit Ph.D.s in drug metabolism, pharmacokinetics, toxicology, and analytical sciences, as well as Pharm.D.s," Dean reports. "We're hiring research nurses, clinicians who have decided that research is more interesting than clinical practice, and biostatisticians. Not many places are training on the regulatory side; people have to move into that from various other disciplines. We hire a lot of people in process chemistry and materials processing. And the informatics part is important. We are a data driven industry; we have a lot of information that has to be processed and evaluated."

Biotechnology firms that have moved into the pharmaceutical realm have their own wide ranging needs for development scientists. "Genencor is at an interesting transition point," Goldsmith says. "The health care division has been increasingly differentiated from the rest of the company. Part of that stems from a greater focus on product stages rather than early basic research. We have been gradually building our postdiscovery activities. Our intention is to cover all aspects of postdiscovery work. One thing that distinguishes us is that we continue to build on the historical strengths of our process development and manufacturing, which many young biotechnology companies can't and don't invest in." Centocor also plans to recruit scientists with several different backgrounds. "We are actively seeking scientific/technical professionals to fulfill needs in process development, clinical research and development, regulatory affairs, clinical manufacturing, technology transfer, and project management," Schmidt says.

Millennium has some highly specific needs. "One of the types we find extremely valuable are the veterinary pathologists who understand how a chemical agent can adversely affect an organism," Smith says. "They need a D.V.M., a residency in pathology, and success in a very rigorous board examination in veterinary pathology that has a passing rate of about 25 percent. We're also looking for people who do modeling in pharmacokinetics; they're hard to come by. People who understand drug metabolism are also hard to find. They train first in chemistry and then in the biology of drug metabolism."


RICH TILLYER

The Quality of Collegiality

What characteristics beyond excellent training do scientists need to succeed in drug development? Biopharmaceutical firms universally seek one key quality in their scientists: collegiality. "Since much of R&D is team based, it is extraordinarily important to be able to work well on teams," Pfizer's Power points out. "Industry is a distinctly different enterprise from academic institutions," Gertz adds. "Everything is focused around teams. The lone entrepreneur won't necessarily succeed in a place like Merck. Scientists need to do that extra amount of work to succeed." Indeed, adds his colleague Tillyer, "Part of our annual appraisal involves teamwork as a key element."

Big pharmas aren't alone in taking that attitude. Biotechnology firms involved in drug development agree that the enterprise is an essentially cooperative one. "One of the key things we look for is dedication to teamwork," Anadys Pharmaceuticals' Xanthopoulos says. "It reflects our core values. Unless you have an interactive, synergistic team that works well together, it will be impossible to discover any molecules. Success depends on individuals and even teams working together."

That fact can baffle scientists fresh from doctoral or postdoctoral work in universities. "As somebody who made a transition from an academic career to a private sector job, I've been pleasantly surprised by the importance of collaboration in industry compared with that in the academic sector," Genencor's Goldsmith says. "Working in a company is much more collaborative, with many parts necessarily working with each other toward combined objectives."

Smith at Millennium points out an essential aspect of teamwork. "It is absolutely critical that the individuals we hire can work in a team environment, communicate complex ideas in a relatively simple way, and understand how to get to the most important information quickly," he says. "The ability to interact with people in other disciplines is absolutely critical. There's a tremendous amount of value in communicating across disciplines. We look for the personalities in a collegial communication-based phenotype. It is important that they be really good scientists. But also they have to be able to communicate with other people."


KLEANTHIS G. XANTHOPOULOS

Crossing Cultures

Communication involves more than crossing disciplines, particularly in global companies. "Ours is not only a team effort; it's an international team effort," explains Dean of sanofi-aventis. "You have to work with different nationalities, different values, and different cultures." Griot of Novartis emphasizes that point. "Development scientists have to have the ability to work in a global cross-cultural matrix," he says. "And of course, they should have the ability to work in virtual multicultural teams."

What else do development scientists need? "We're looking for leadership," Griot continues. "If scientists want to succeed, they need to be able to manage people effectively," Merck's Gertz adds. "That's generally not something scientists are trained to do. We try to train them for it."

Centocor's Schmidt summarizes the qualities that corporations expect in their development scientists. "Beyond technical expertise," he says, "the critical skills for which we look include communication skills, the ability to collaborate in a team-based environment, the ability to manage complexity and change, people development skills, innovative thoughts, and personal accountability to drive for results." Vertex's Patrick emphasizes that recruiting for drug development involves intangible qualities as well as measurable qualifications. "The technical competencies and advanced degrees are important," he says. "But it is the behavioral traits that get the new graduate a job."

A former science editor of Newsweek, *Peter Gwynne (pgwynne767@aol.com) covers science and technology from his base on Cape Cod, Massachusetts, U.S.A.*

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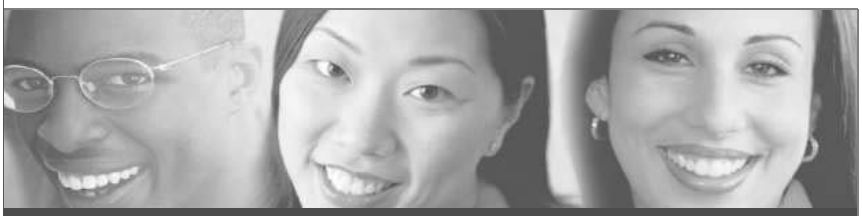

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Department of Cardiovascular Disease

Senior/Principal Scientist. Lead a research group, act as a project champion, and represent the group in multidisciplinary project teams. Requires a background in biology or medicine with a strong track record in cardiovascular disease research, particularly Hypertension and End Organ Damage (renal and/or cardiac failure). Prefer a candidate with several years' experience in the pharmaceutical or biotech industry. **Job Code RD5005**

Scientists. Develop, evaluate and establish preclinical in vivo models, along with biomarkers focused on hypertension and renal/cardiac damage. Must be a Bachelor's/Master's-level scientist skilled in cardiovascular in vivo physiology/pharmacology. Experience in sterile surgical procedures a plus. **Job Code RD4105.**

Department of Drug Discovery Support

Senior Scientist, Pharmaceutics. Develop preclinical formulations to support pharmacology/PK/Toxicology studies, solution/solid state characterization, and high-throughput property assays. A history of contributing to peer-reviewed journals is required. This is a team-oriented role in a fast-paced, multidisciplinary environment. Requires Ph.D. in Pharmaceutics (or related field) and 3 years' proven drug discovery support experience. **Job Code RD0205**

Principal Scientist, Pharmacokinetics. Design and conduct Pharmacokinetic studies, interpret results and provide key information for characterization and selection of drug development candidates. Must have broad knowledge of DMPK and experience with biotransformations, drug-drug interactions, in vitro/in vivo correlations, Pharmacokinetic-Pharmacodynamic relations and competency in quantitative Bioanalytical Chemistry, including LC/MS/MS. A Ph.D. in Pharmacokinetics and 5 years in DMPK at a pharmaceutical company are required. **Job Code RD2105**

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InterGenetics has identified gene-gene and gene-environmental interactions that have been applied to the construction of predictive cancer risk models for breast cancer. Current analyses rely on computer intensive iterative resampling and data randomization to identify input variables. The candidate selected for this position will work with a team to provide insights into cancer risk model development methods and possibly take the lead on developing future models for other cancer risk tests.

Applicants must have a Ph.D. in Statistical Genetics or related discipline and 5+ years post-graduate work in the field of cancer risk and/or other complex disease modeling. An extensive genetics background and working knowledge of genetics is required.

MOLECULAR BIOLOGIST / RESEARCH LABORATORY MANAGER

The successful candidate will: participate in research and development to identify, improve and implement a genetics-based cancer risk model through to the commercial environment; contribute to the improvement of assay development and new platforms; write grants for additional research projects; writing manuscripts for peer reviewed publication; and contribute to new research in the development of follow-on tests and pharmacogenomic and targeted therapeutic research.

Applicants must have a Ph.D. in molecular biology with 5+ years research experience in genetic predisposition to disease, preferably cancer. The ideal candidate will have excellent writing skills and appropriate publications record for experience. This position will require creative and critical thinking and problem solving. Experience in high throughput PCR based assay development and validation required.

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EOE

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We have the following exciting opportunities in our **Foster City** location.

Director/Senior Director, Biology (HCV)

(Job # RF1-410)

The successful candidate will direct Biology research in the field of Hepatitis C Virus (HCV) and will report to the VP, Biology. He/she will propose new targets for drug discovery and initiate and direct scientific research for the discovery of drug candidates in the field of HCV. He/she will guide all HCV laboratory experimentation, including development and execution of high-quality primary and secondary Biology assays. He/she will manage a group of 9 biologists, including 3 Ph.D. level scientists. He/she will participate in, or lead, interdisciplinary drug discovery teams and manage external academic and industrial collaborations as required. Publication and presentation of company research is expected.

The candidate will likely be a recognized leader in HCV research or a related field. The position typically requires a Ph.D. with a minimum of 10 years of relevant experience. Broad expertise and knowledge of molecular and cell biology, virology and biochemistry/enzymology are required; industrial experience and demonstrated success in drug discovery are preferred. A strong performance focus and commitment to integrity are expected, as are analytical and problem-solving skills, communication and team-building skills.

Research Scientist/Senior Research Scientist, Biology (HCV)

(Job # BF5-280)

The successful candidate will join a drug discovery team focused on the discovery of drug candidates for the treatment of HCV. He/she will participate in the biological profiling of drug candidate molecules by developing and employing state of the art in vitro efficacy and drug resistance assays. Additional responsibilities will include identifying new antiviral and other therapeutic targets, designing and developing novel biological assays, and performing in vitro mechanistic pharmacology studies. Publication and presentation of company research is expected.

This position typically requires a Ph.D. with 2-5 years of relevant postdoctoral experience. Broad expertise and knowledge of molecular and cell biology, virology and biochemistry techniques is expected. Experience in enzymology and assay development is a strong plus. A solid publication record, strong oral and written communication skills, and ability to work in multidisciplinary teams are required.

Research Associate/Senior Research Associate, Biology (HCV)

(Job # BF5-265)

The successful candidate will join a drug discovery team focused on the identification of new therapies for HCV. His/her primary duty will be antiviral evaluation of corporate compounds in cell-based assays. They will also contribute to the development and optimization of novel cell-based assays and have the opportunity to learn enzymology. Additional duties include presentation of data at group meetings, data management, and general laboratory and equipment maintenance.

The position requires a Bachelor's or Master's degree in Life Sciences, along with cell culture experience. Molecular biology skills (DNA sequencing, PCR, mutagenesis, and cloning) and Virology experience are preferred. Good oral and written communication skills, and ability to work in multidisciplinary teams are required.

Research Scientist, Biology (HIV)

(Job # RF5-227)

The successful candidate will join a drug discovery team focused on the identification of new therapies for Human Immunodeficiency Virus (HIV). He/she will participate in the biological profiling of drug candidate molecules by developing and employing state of the art in vitro efficacy and drug resistance assays. Additional responsibilities will include identifying new antiviral and other therapeutic targets, designing and developing novel biological assays, and performing in vitro mechanistic pharmacology studies. Publication and presentation of company research is expected.

The position requires a Ph.D. with a minimum of 2 years of relevant postdoctoral experience. Broad expertise and knowledge of molecular and cell biology, virology and biochemistry techniques including assay development is expected. Experience in enzymology is a plus. A solid publication record, strong oral and written communication skills, and ability to work in multidisciplinary teams are required.

Research Scientist/Senior Research Scientist, Biology (Biophysics)

(Job # BF5-140)

The successful candidate will be a key member of the Protein Chemistry Group responsible for the development and implementation of methods to characterize the interaction of small molecule ligands with protein targets supporting the development of drug screening assays, crystallography, High Through-put Screening (HTS), and rational drug design.

The position requires a Ph.D. with 2-4 years of relevant postdoctoral experience. Industrial experience preferred. Must have demonstrated expertise in the biophysical and structural characterization of proteins. Experience in using biophysical instrumentation to study the interaction of organic ligands with protein targets and working knowledge of mass spectrometry, circular dichroism, analytical centrifugation, micro-calorimetry and/or binding kinetics highly preferred. A solid publication record, strong oral and written communication skills, and ability to work in multidisciplinary teams are required.

Senior Research Scientist/Principal Scientist, Clinical Virology

(Job # BF5-279)

The successful candidate will support the preclinical and clinical development of anti-HCV compounds and investigate resistance to these compounds at the cellular and molecular level. He/she will define the clinical resistance profile of anti-HCV compounds in development. Publication and presentation of company research at scientific meetings, in publications and for regulatory submissions is expected.

The position requires a Ph.D. or M.D. degree with a minimum of 6 years of relevant experience, preferably in the field of HCV. Extensive cell biology, molecular biology, enzymology and virology laboratory experience is expected, as are strong oral and written communication skills. Regulatory experience is also desirable.

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Focusing Science to ADVANCE MEDICINE



Scios Inc., headquartered in Fremont, California, is changing the way heart failure is treated. We are developing new and exciting therapeutics for cardiovascular disease, inflammatory disease and cancer. Our winning combination of integrated research, clinical experience, and passionate commitment to patients with unmet medical needs is making a difference in the management of disease. Innovation, collaboration and education are the cornerstones of Scios' culture.

We provide mentoring and career development opportunities throughout our organization. Our ability to grow as a company depends on finding and nurturing individuals with entrepreneurial spirit and passion to change the way patients are treated.

To support our continued growth, we seek candidates in the following areas:

CLINICAL RESEARCH

- Clinical Data Coder
- Clinical Research Associates
- Directors
- Lead - Clinical Data Mangement
- Manager, Supervisor - Clinical Supplies
- Managers
- Scientist

RESEARCH

- Associate Director, Bioanalytics
- Post Doc - Cell Biology
- Managers - Bioanalytics
- Medical Writers
- Research Associates, Formulations, HTS, Analytical Chemistry
- Scientists - Genomics, Informatics Toxicology, Analytical Chemistry, Pharmacokinetics

MEDICAL AFFAIRS

- Associate Director
- Directors
- Managers
- Medical Education
- Medical Writer
- Manager, Medical Education
- Senior Biostatistian, Lead, Associate Director - Biostatistics

REGULATORY AFFAIRS

- Associates
- Directors
- Managers

QUALITY CONTROL/ QUALITY ASSURANCE

- Analysts
- Specialist

For more information about us, to view a complete job description, and to apply online, please visit us at:

www.sciosinc.com

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Positions @ NIH
THE NATIONAL INSTITUTES OF HEALTH

AFFECT AND SOCIAL BEHAVIOR PROGRAM

The National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, anticipates an opening for a Program Officer at its Rockville, MD site to guide and manage a grants program supporting basic research on the fundamental principles and mechanisms of affect, social behavior and social cognition in both humans and animals. Topic areas within this grants program include the basic processes, development, and regulation of emotion, mood, agonistic and affiliative behaviors and social communication. The program also supports work on fundamental mechanisms of social information processing. The Program Officer will be responsible for maintaining and further developing an innovative program in this research area that includes behavioral science and behavioral and systems neuroscience approaches. Responsibilities will include administering and managing an extramural portfolio of research awards, interacting with researchers and related programs at NIMH, NIH, and other funding agencies, and developing new research initiatives. Candidates must be U.S. citizens and have a Ph.D., M.D., or equivalent degree. Prior research experience spanning the fields of behavioral science and behavioral or systems neuroscience is preferred. Experience in two or more of the following research areas is desirable: behavioral science, social neuroscience, affective neuroscience, and behavioral neuroscience. At a minimum, candidates should be able to demonstrate their willingness and capacity to expand their current area of expertise to cover this broad domain of research. The position requires working both independently and collaboratively. Strong organizational and oral and written communication skills are also required. Salary will be commensurate with experience. Send a letter of interest and CV by email to **Kevin Quinn, Ph.D. at kquinn@mail.nih.gov (Tel: 301-443-1576) by September 23rd, 2005.** With nationwide responsibility for improving the health and well being of all Americans, the Department of Health & Human Services oversees the biomedical research programs of the National Institutes of Health **(http://www.os.dhhs.gov)**

CIRCADIAN RHYTHMS, SLEEP, AND REGULATION OF BEHAVIOR PROGRAM

The National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, anticipates an opening for a Program Officer at its Rockville, MD site to guide and manage an extramural grants program supporting basic research on the fundamental principles and mechanisms of biobehavioral regulation. Topic areas within this grants program include the basic processes, development, and regulation of circadian rhythms, sleep, motivation, feeding, play, and aggression. The interaction of nervous, endocrine and gene systems with cognition, biological rhythms, stress, and social variables is a major focus in this research portfolio. Analysis of sex differences and basic functional neuroanatomy are also important components of the program. The Program Officer will be responsible for maintaining and further developing an innovative program in this research domain that includes behavioral science and behavioral and systems neuroscience approaches. Responsibilities will include administering and managing an extramural portfolio of research awards, interacting with researchers and program officers for related programs at NIMH, NIH, and other funding agencies, and developing new initiatives. Candidates must be U.S. citizens and have a Ph.D., M.D., or equivalent degree. Prior research experience spanning the fields of behavioral science and behavioral or systems neuroscience is preferred. At a minimum, candidates should be able to demonstrate their willingness and capacity to expand their current area of expertise to cover this broad domain of research. The position requires working both independently and collaboratively. Strong organizational and oral and written communication skills are also required. Salary will be commensurate with experience. Send a letter of interest and CV by email to **Kevin Quinn, Ph.D. at kquinn@mail.nih.gov (Tel: 301-443-1576) by September 23rd, 2005.** With nationwide responsibility for improving the health and well being of all Americans, the Department of Health & Human Services oversees the biomedical research programs of the National Institutes of Health **(http://www.os.dhhs.gov).**

Tenure-Track Position

With nation-wide responsibility for improving the health and well being of all Americans, the Department of Health and Human Services (DHHS) oversees the biomedical research programs of the National Institutes of Health (NIH) and those of NIH's research Institutes.

The National Institute of Mental Health (NIMH), a major research component of the NIH, and the DHHS, is recruiting for a tenure-track neuroscientist in the new Genes, Cognition and Psychosis Program (GCAP). With a complimentary budget and staff, the individual selected for this position will be expected to establish an independent research program focused on cellular and molecular neuroscience relevant to schizophrenia.

The successful individual must possess an M.D. and/or Ph.D. degree, and experience in cellular biochemistry, imaging, or electrophysiology is preferable. At least five years of relevant research experience is required.

There are strong interactions among the independent research group, and the position offers unparallel opportunities for interdisciplinary collaboration with the scientists within GCAP and throughout the NIH. Salary is commensurate with experience and accomplishments, and a full Civil Service package of benefits (including retirement, health, life, and long term care insurance, Thrift Savings Plan participation, etc.) is available.

The strong scientific environment and outstanding equipment resources at NIH makes this a unique opportunity for an outstanding scientist. Interested candidates should send curriculum vitae, statement of research interests, accomplishments and future goals, and three letters of recommendation to the **Chair, Search Committee for a Tenure Track Investigator in the area of Cell Biology, National Institute of Mental Health, Building 10, Room 4N-222, 9000 Rockville Pike, Bethesda, MD 20892, or by email to: steyerm@mail.nih.gov by July 31, 2005.**

DIRECTOR'S FELLOWSHIP

The National Center for Complementary and Alternative Medicine (NCCAM) seeks outstanding candidates for the NCCAM Director's Fellowship at the National Institutes of Health (NIH) in Bethesda, Maryland.

The fellowship includes full salary, benefits, professional travel, and research support for 2-3 years. The fellow will undertake CAM related clinical, translational, and/or laboratory research in the NIH's intramural program. The candidate will be a bridge between NCCAM and the Institute where the work will be performed. The NIH intramural program provides state-of-the-art research facilities, access to the extensive clinical research facilities of the NIH Clinical Center, and a collegial and nurturing work environment.

Applicants must possess an MD, DO, PhD, DC, ND, or other equivalent degree, and have a record of excellence in clinical and/or laboratory research. Preference will be given to candidates with specific research interests and experience in complementary and alternative medicine, particularly related to neuroscience (imaging), social and behavioral sciences, pharmacology, or immunology. Applications from women and minorities are encouraged.

Applicants should forward their CV, bibliography, names of three references, and a one-page cover letter defining their area of interest and summarizing their scientific interests and experience to:

NCCAM Director's Fellowship Search Committee, NCCAM, 31 Center Drive, Room 2B-11, MSC 2182, Bethesda, MD 20892; (Fax 301-402-4741) (Email: nccamdirector-r@mail.nih.gov); Refer to Announcement NCCAM-PWF05; Deadline for receipt of applications is September 16, 2005.

This fellowship is provided through the generous support of The Prince of Wales Foundation, a charity that supports research in complementary and rnative medicine and integrative health care.

WWW.NIH.GOV

Center for Scientific Review

MUSCULOSKELETAL SCIENTIST

We are seeking an exceptionally qualified scientist, with doctorate level training and independent research experience in musculoskeletal physiology, pathology and/or structure, to join a team of Scientific Review Administrators (SRAs) to help shape the future of scientific review. The incumbent will be responsible for the initial administrative and scientific review of musculoskeletal research grant applications and will possess an M.D. or Ph.D. degree (or have equivalent training and experience) with independent research and administrative experience and a publication record. The position is in the Musculoskeletal, Oral and Skin Sciences Integrated Review Group (IRG). This IRG is responsible for the merit evaluation of the full range of basic, translational and clinical studies dealing with the array of hard and soft tissues (including bone, cartilage, muscle, skin and dental/oral and craniofacial structures) associated with the musculoskeletal system. For additional information on the IRG please see our web site, at:
http://www.csr.nih.gov/review/MOSSIRG.HTM

Salary is commensurate with research experience and accomplishments, and a full Civil Service package of benefits (including retirement, health, life and long-term care insurance, Thrift Savings Plan participation, etc. is available. For additional information on this position, and for instructions on submitting your application, please see our website, at: **www.csr.nih.gov . The closing date for this position is July 8, 2005.**

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

CELL BIOLOGY AND METABOLISM BRANCH
CELL CYCLE REGULATION IN DEVELOPMENT

A **postdoctoral position** is currently available to study cell cycle regulation during Drosophila oogenesis. Current projects include using genetic and molecular approaches to identify the pathways that control meiotic entry and the maintenance of the prophase I meiotic arrest of the oocyte.

The National Institutes of Health provides excellent training opportunities including access to genomic and proteomic core facilities and state-of-the-art imaging. The Bethesda campus is located in the Washington DC metropolitan area and offers a culturally rich and diverse environment.

Candidates should have less than five years postdoctoral experience and hold either a Ph.D. or M.D., or equivalent. Positions are available for a minimum of 2 years and may be extended for additional training. Salary is commensurate with experience ($38,500 per annum minimum). A strong background in molecular biology and genetics is required. Please send your CV and contact information for three references by email to:

Dr. Mary Lilly, NIH/NICHD, Cell Biology and Metabolism Branch, Bldg. 18, Rm 101, Bethesda, MD 20892;Email: mlilly@helix.nih.gov
http://dir2.nichd.nih.gov/nichd/cbmb/uccr/uccr.html

NATIONAL CANCER INSTITUTE

Be an NCI Cancer Prevention Fellow

The Cancer Prevention Fellowship Program provides training for individuals from the health professions and biomedical sciences to become leaders in the field of cancer prevention and control. The Program is sponsored by the Department of Health and Human Services, the National Institutes of Health, the National Cancer Institute (NCI), and the Division of Cancer Prevention.

What will I get out of the program?
- Master of Public Health (M.P.H.) degree
- NCI Summer Curriculum in Cancer Prevention
- Mentored research opportunities t the NCI or at the Food and Drug Administration (FDA)
- Professional development and leadership training

What areas of cancer prevention research are available?
- Chemoprevention
- Clinical cancer prevention
- Development and research-related review of drugs, biologics, or medical devices
- Epidemiology (environmental, genetic, molecular, nutritional)
- Ethics and evidence-based decision making
- Laboratory-based research
- Screening and early detection
- Social and behavioral research
- Statistical methodology

Am I eligible?
You must have a doctoral degree (M.D., Ph.D., J.D., or equivalent). Foreign education must be comparable to that received in the United States.

You must also be a citizen or permanent resident of the United States at the time of application (September 1).

How long is the program?
The typical duration is 3 years (year 1: M.P.H.; years 2-3: NCI Summer Curriculum in Cancer Prevention and mentored research).

How do I obtain more information?
Visit our website **http://cancer.gov/prevention/pob** or request a catalog.

To receive a catalog*, contact:
Douglas L. Weed, M.D., M.P.H., Ph.D.
Director, Cancer Prevention Fellowship Program
National Cancer Institute
6130 Executive Boulevard (EPN)
Suite 321, MSC 7361
Bethesda, MD 20892-7361

** Please provide home address, telephone, e-mail, and where you heard about the Program.*

How do I apply?
Apply online at **http://cancer.gov/prevention/pob** or send your application materials directly to the Cancer Prevention Fellowship Program Director, as described on our website and in our catalog.

When are applications due?
Applications are due September 1 for entry into the Program the following July 1.

Further inquiries:
Program Coordinator
Cancer Prevention Fellowship Program
Phone (301) 496-8640
Fax (301) 402-4863
E-mail: cpfpcoordinator@mail.nih.gov

DHHS, NIH, AND NCI ARE EQUAL OPPORTUNITY EMPLOYERS

Selection for these positions will be based solely on merit, with no discrimination for non-merit reasons, such as race, color, gender, national origin, age, religion, sexual orientation, or physical or mental disability. NIH provides reasonable accommodations to applicants with disabilities. If you need reasonable accommodation during any part of the application and hiring process, please notify us. The decision on granting reasonable accommodation will be handled on a case-by-case basis.

PURDUE UNIVERSITY

Director
Birck Nanotechnology Center

The Birck Nanotechnology Center is housed in a new $60M building that will open in the summer of 2005. The 187,000 ft2 facility has class 10, 100 and 1000 clean rooms as well as a biological clean room and NIST A-1 low vibration laboratories. Currently the Center has 129 faculty members from 27 different departments. See **www.nano.purdue.edu** for more details on the facilities, the Birck Center, and Discovery Park.

Purdue University seeks an individual with vision and demonstrated leadership for the position of Director of the Birck Nanotechnology Center at Discovery Park on the West Lafayette, Indiana campus (**www.purdue.edu/dp**). The Director must be a well-established investigator with a distinguished record of scholarly and scientific accomplishment in interdisciplinary nanotechnology research. Administrative experience in the leadership of multi-investigator research programs and industrial partnerships is desirable. Responsibilities include the development of the Center's expanding vision, mission, and role on campus.

The Director reports to the Executive Director of Discovery Park and works closely with academic Deans, Department Heads, Discovery Park researchers, and the Birck Nanotechnology Center leadership in the administration of the program. The position carries an appointment as a Professor in the Science or Engineering department or departments appropriate to the individual's research and teaching expertise. The position is supported by an endowed chair.

Applicants should submit, via email to **dstarewi@purdue.edu**, a letter of interest that outlines qualifications for the position, curriculum vitae, and the names of three references (including their postal and e-mail addresses, and phone numbers). Nominations are also welcome and should be emailed for routing to the search committee. Review of applications will begin immediately and will continue until the position is filled.

Purdue University is an Equal Opportunity/Equal Access/Affirmative Action Employer fully committed to achieving a diverse workforce. Women, minority applicants, and dual career couples are encouraged to apply.

ASSOCIATE TO FULL PROFESSOR OF MEDICAL SCIENCE (tenure track)
PROGRAM IN PUBLIC HEALTH
BROWN MEDICAL SCHOOL

A senior faculty position (Associate or Full Professor) in Behavioral and Social Sciences is available in the Department of Community Health at Brown University beginning January 2006. The position is part of a multi-year expansion plan for Public Health at Brown which will entail recruiting up to 17 new tenure-track positions.

Minimum requirements include a national or international (for full professor) reputation in an area of research, an independently funded program of research and a proven track record of leadership in research and training. Doctoral degree in a Behavioral and/or Social Science (or equivalent) is required.

Preferred characteristics include experience leading collaborative, interdisciplinary research. Areas of special interest include multi-level research, addictions, application of theory, community-based research, dissemination, and/or international issues.

Interested applicants should send a letter of application, a curriculum vitae and names and contact information of five referees, who may be contacted, to: **Peter M. Monti, Ph.D., Chair of Behavioral and Social Sciences Search Committee, Brown University, Department of Community Health, Box G-BH, Providence, RI 02912**. **Chair-BSS_Community_Health@Brown.edu.**

Brown University is an Affirmative Action/Equal Opportunity Employer and actively solicits applications from women and minority group organizations.

USDA ARS Agricultural Research Service
www.ars.usda.gov

Research Leader, Crop Improvement and Utilization
USDA/ARS Western Regional Research Center
Salary Range of $96,300.00 - $140,300.00 PA

The USDA, Agricultural Research Service, is seeking a highly motivated person to fill a leadership position at the Western Regional Research Center. The research center is located in Albany, California, in the San Francisco Bay Area. The **Research Leader** in the **Crop Improvement Utilization Research Unit** will head a 40-person group whose mission is to apply biochemical and molecular/genetic approaches, including genomics and proteomics, to enhance the utilization of plants for food and non-food uses. Research goals are to **(1) develop new approaches improving crop plants that minimize risks to people and environment; (2) improve wheat quality and agronomic characteristics; (3) reduce alkaloid levels and post-harvest pest and disease problems in potato; (4) develop new crops to serve as domestic sources of rubber; and (5) modify lipid composition in economically important oilseed crops.** As Research Leader, the successful candidate will guide the Unit's research direction, enhancing research capability and productivity, fostering collaboration within ARS and with university and industry partners, managing financial and human resources, and identifying opportunities for Unit personnel.

The successful candidate also is expected to conduct an active and high quality research effort related to the goals of the Unit, such as in the application of plant molecular biology to improve latex-producing crops and to reduce risks associated with plant transformation. The ideal candidate will have a strong scientific record, leadership experience, and enjoy working in a collegial atmosphere to provide vision to a state-of-the-art and growing research program.

For application directions, see **www.afm.ars.usda.gov/divisions/hrd/index.html**. Announcement Number is **ARS-X5W-0297**. This announcement is open until filled. For questions you may contact **Dr. James Seiber** at **510-559-5600** or e-mail: **jseiber@pw.usda.gov**.

USDA, ARS is an Equal Opportunity Employer and Provider.

DIRECTOR, CENTER FOR BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

National Institute of General Medical Sciences (NIGMS)
National Institutes of Health (NIH)
Department of Health and Human Services (DHHS)

The Challenge: A significant challenge for the biomedical research community is the integration of the vast amount of accumulating scientific data in order to develop predictive understanding of basic biological processes. The ability to meet this challenge will be critically dependent on advances in bioinformatics and computational biology. To this end, in 2001, NIGMS established a Center that is responsible for stimulating and funding research in areas of importance for NIGMS. The Center supports research on bioinformatics, databases, and data mining; on modeling of complex biological systems; on algorithmic development and software engineering; and on mathematical biology, among other areas. In addition, the Center is responsible for managing the NIH Biomedical Information Science and Technology Initiative (BISTI), an agency-wide effort to stimulate and coordinate use of computer science and technology to address problems in biology and medicine. Finally, the Center plays a major role in coordinating and directing the Bioinformatics and Computational Biology component of the NIH Roadmap for Medical Research. **The institute is seeking a leader in this field to direct the Center and the BISTI efforts, and to coordinate the work of both with other interested federal agencies and the broader scientific community.** Information about the Center and BISTI is available at: **http://www.nigms.nih.gov/about_nigms/cbcb.html**.

Position Requirements: Candidates must have an M.D., Ph.D. or equivalent degree in a field relevant to the position. The ideal candidate will have considerable research experience demonstrating a strong understanding of both computation and biological issues. In addition, candidates should possess recognized research management and leadership abilities. The position will be filled under Title 42, offering a competitive salary commensurate with qualifications and experience, within the range of $125,304 to $175,700. A recruitment or relocation bonus may be available. Relocation expenses will be paid. An intramural research program is possible, subject to negotiation.

How to Apply: The official vacancy announcement is available at: **http://www.nigms.nih.gov/about/job_vacancies.html**. To be considered for this position, send to the address below a CV, bibliography, the names and contact information of 4 references, and **a "vision statement," not to exceed 3 pages, that presents your views of the most significant challenges and opportunities in bioinformatics and computational biology relevant to NIGMS that you would seek to address should you be selected for this position.**

nigmsjobs@mail.nih.gov or FAX to **301-451-5686**
Applications must be received by midnight on the closing date: **Thursday, August 4, 2005**

You may contact **Stephanie Klingenberg**, Human Resources Specialist, with questions about this vacancy on **301-594-2233**.

DHHS, NIH, and NIGMS are Equal Opportunity Employers.

CHAIR
Department of Biochemistry and Molecular Medicine

The University of California, Davis, School of Medicine seeks a visionary academic leader to lead the Department of Biochemistry and Molecular Medicine. The successful candidate must have the ability to anticipate change and implement initiatives to meet the challenges of academic medicine and managed care, to work cooperatively and collegially within a diverse environment, and excellent interpersonal skills to build and maintain relationships with the academic community. Candidates must have a Ph.D. in Biochemistry and Molecular Medicine or a related field, a distinguished record in research, teaching, and administration, and must meet requirements for appointment as Associate or Full Professor in one of the University of California's academic series. It is anticipated that the chair will devote approximately 50% of his/her effort to administrative duties and university service, 30% to research, and 20% to teaching.

The Department of Biochemistry and Molecular Medicine has full-time Ph.D. faculty scientists in a variety of research areas. Educational programs include a 20-person residency program and specialty fellowships.

Send curriculum vitae, statement of administrative, clinical and research background and names of at least five references to: **Biochemistry and Molecular Medicine Chair Search Committee**, via e-mail at **janice.weir@ucdavis.edu**, or via regular mail to **Janice Weir, c/o Office of Academic Affairs, School of Medicine, University of California, Davis, Medical Center, PSSB Suite 2500, 4150 V Street, Sacramento, CA 95817.**

For full consideration, applications must be received by **September 30, 2005**. The position will remain open until filled through July 1, 2006.

The University of California is an Affirmative Action/ Equal Opportunity Employer.

Great Lakes Research Investigator Program
School of Natural Resources & Environment
University of Michigan

The University of Michigan's School of Natural Resources & Environment, with NOAA's Great Lakes Environmental Research Laboratory and the USGS Great Lakes Science Center, is seeking qualified candidates for two-year Joint Research Investigator positions in the following areas. Renewal for second two-year appointments are possible provided satisfactory performance and proposal writing success.

Environmental Toxicologist Studies of the potential effects of cyanobacterial toxins on human health and food-webs

Beach Bacterial Contamination Studies of interactions of hydro-meteorological and ambient conditions with indicator bacteria, such as E. coli or enterococci

Watershed Hydrology Spatially explicit models for watershed and open lake forecasts of water and material (nutrients, sediment, pollutants, etc) movement

Statistical Modeling and Forecasting Use of large spatial and temporal databases for statistically forecasting Great Lakes conditions (e.g. algal blooms, beach closings, physical hazards, fish recruitment, water quality)

Fish thiamine deficiency Identification of trophic pathways associated with Thiamine Deficiency Complex in Great Lakes salmonids and characterization of fish diets using fatty acid profile analysis.

Ecological Toxicology Studies to model and assess impacts of toxicants at the individual, population, and ecological levels of the Great Lakes

Near-shore Coastal Hydrodynamics and Particle Transport Studies of how hydrodynamic processes in the near shore zone affect the transport of dissolved and suspended materials for the purpose of operational predictions of near shore water quality

Coastal Observation Apply real-time buoy-deployed chemical, biological, physical sensors and remote sensing to study and forecast Great Lakes environmental processes.

Fish Ecology Study the distribution, habitats, feeding ecology and bioenergetics of fishes in the Great Lakes and Chesapeake Bay using underwater acoustics and spatial bioenergetics modeling

For more information, including a full description of these areas of interest and individual contacts, visit www.ciler.snre.umich.edu. To apply, send CV and statement of research objectives by July 22, 2005 to Dr. Donald Scavia, 520 Dana Bldg., 440 Church St., University of Michigan, Ann Arbor, Michigan 48109-1041 or scavia@umich.edu.

NOAA

NATURAL RESOURCES AND ENVIRONMENT
UNIVERSITY OF MICHIGAN

USGS
science for a changing world

The University of Michigan is an equal opportunity/affirmative action employer.

Assistant/Associate Professor

Forest Biologist

The Faculty of Environmental & Forest Biology of the State University of New York, College of Environmental Science & Forestry, in Syracuse, invites applications for an Assistant or Associate Professor position in Forest Biology in support of its program in Forest Health. A Ph.D. in Biology, Forestry, Environmental Science, or a related discipline; as well as a record of publication, grantsmanship, and independent research are required. The successful candidate is expected to develop a strong, internationally recognized, externally funded, and interdisciplinary research program that addresses the biological, physiological, and/or environmental factors that affect the health of forests. Such factors include but are not limited to pathogens, pests, climate change, pollutants, invasive species, and/or management activities. We specifically seek both an outstanding researcher and an enthusiastic teacher. Teaching will include both undergraduate and graduate level courses. Effective collaboration with colleagues in forest health, and in our other programs in environmental biology, wildlife science, aquatic & fisheries science, conservation biology, biotechnology, natural history & interpretation, forestry, and/or chemistry; as well as some outreach activity are expected.

Applicants should send statements of research interests, teaching philosophy, CV, and a list of three references directly to: ATTN: **Forest Biologist Faculty Position, Office of Human Resources, SUNY-ESF, 217 Bray Hall, 1 Forestry Drive, Syracuse, NY 13210-2778, USA.** Position is open until filled, but for full consideration materials should be received by SEPTEMBER 20, 2005.

Visit ESF on the web at **www.esf.edu**

SUNY-ESF is an Equal Opportunity/Affirmative Action employer.

OHSU | OGI SCHOOL OF SCIENCE & ENGINEERING

The Gordon and Betty Moore Chair
OGI School of Science and Engineering
Oregon Health and Science University

Distinguished applicants are encouraged to apply for the endowed **Gordon and Betty Moore Chair** at OHSU's OGI School of Science and Engineering. As an integrated part of the only academic health center in Oregon, OGI is uniquely positioned to bring advanced science, computational and engineering methodologies to bear on complex problems of human and environmental health. For more information about OGI and OHSU, please visit our website at www.ogi.edu.

We seek an investigator whose established research program(s) at the interface between advanced technology and human and environmental health will complement the existing strengths of our faculty. We are particularly interested in candidates whose research, interdisciplinary interests, vision, and leadership qualities will result in the creation of a world-class nanobiotechnology research and graduate education center that will leverage high-level collaborations with OHSU's research and patient care communities as well as with other institutions in Oregon.

Qualified applicants are encouraged to submit a letter of application, a curriculum vitae and a summary of research and educational objectives to:

Dr. William H. Glaze, Associate Dean
Gordon and Betty Moore Chair Search Committee
OGI School of Science and Engineering
Oregon Health and Science University
20000 NW Walker Road, Mail Code OGI-801
Beaverton, OR 97006-8921

Electronic submissions may be sent to: **hendricc@ohsu.edu**

OHSU is an Affirmative Action, Equal Opportunity Institution.

UNIVERSITY OF OXFORD

Life and Environmental Sciences Division

School of Geography and the Environment

University Lecturership in Biodiversity and Conservation

in association with Oriel College
(the Jackson Senior Research Fellowship)

The School of Geography and Environment and the Environmental Change Institute propose to appoint a University Lecturer in Biodiversity and Conservation. The Lecturership is tenable from September 2005 and includes appointment to the Jackson Senior Research Fellowship at Oriel College. The combined college and university salary will be according to age on a scale up to £45,707 p.a. (as at 1st August 2004).

The appointee will be required to engage in research which will contribute to the department's research reputation; to teach, supervise and examine undergraduate and graduate students; and to contribute to administration in college and department. The successful appointee will be expected to contribute to the research programmes of the ECI, particularly in Biodiversity and Conservation, and those of the School at large. Teaching will be centred on the MSc in Environmental Change & Management, although the appointee will also be expected to contribute to other cognate programmes within the School. The appointee will be expected to supervise DPhil students.

The Jackson Senior Research Fellowship at Oriel College is intended to foster postgraduate research on the environment within the College.

Further particulars are available from http://www.admin.ox.ac.uk/fp/ or from Professor G Clark, Head of the School (e-mail gordon.clark@ouce.ox.ac.uk). Informal enquiries should be addressed to either Professor Diana Liverman, Director of the Environmental Change Institute (diana.liverman@eci.ox.ac.uk), or Professor Clark.

Applications (eight copies except from candidates overseas who need send only one), including a curriculum vitae, a list of principal publications, and the names of two referees, should be sent to Professor Gordon L Clark, Head, School of Geography and the Environment, Mansfield Road, Oxford OX1 3TB for receipt no later than 29th June 2005.

Research Fellow(s)

Grade D31 Research staff (RS1A). Salary: £19,460 to £29,128 p.a.

Applications are invited for two research fellows to work on a project funded by the Economic and Social Research Council as part of a wider Gender Network initiative. The aim of the project is to examine the links between migrant labour and gendered patterns of labour market participation in the health service and the hospitality industry. Applicants should have a postgraduate degree in economic geography or a related field. Experience in using qualitative research methods (including semi-structured interviewing) is important, although other methods of data collection will also be used.

Both posts are available from October 2005 or sooner and are for 24 months. They will involve working on all aspects of the research which will be directed by Professor Linda McDowell (OUCE, Oxford) and Dr Claire Dwyer (Geography, UCL). The posts are based in the School of Geography at Oxford and so travel to and from London will be necessary during the interviewing stage of the work.

Further details and an application form are available from Mrs Jan Burke and online at www.geog.ox.ac.uk/ Informal enquiries may be made to Professor McDowell (linda.mcdowell@ouce.ox.ac.uk). Closing date for receipt of applications is 29th June 2005.

The University is an Equal Opportunities Employer.

Western

Dean of Science

Applications and nominations are invited for the position of Dean, Faculty of Science, The University of Western Ontario. The appointment, to be effective 1 July 2006, is for a period of five years, renewable. Candidates should have:

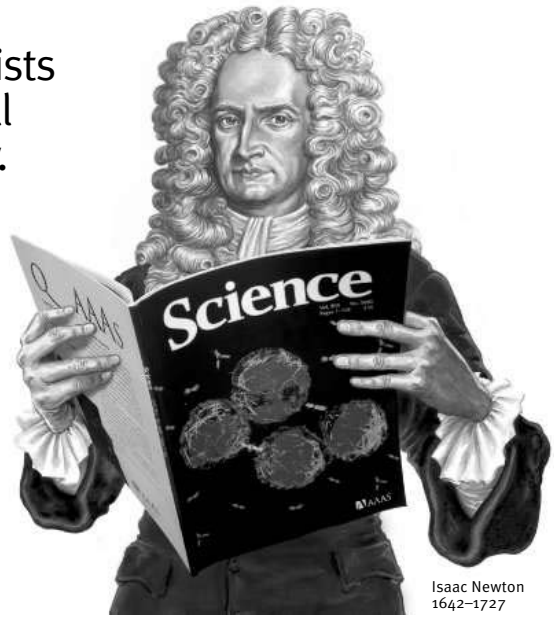
- an established reputation as an academic researcher and teacher;
- a vision for the study of science at a major Canadian research university;
- the administrative, interpersonal, and communications skills to provide dedicated and dynamic leadership for the academic programs and to capitalize on the research strengths within the Faculty;
- the energy and commitment to sustain and expand the significant research investments made in the Faculty by the Canada Foundation for Innovation and related programs.

The University of Western Ontario, including its three Affiliated University Colleges, has a total enrolment of approximately 34,000 full-time equivalent students in graduate, undergraduate and professional programs. The Faculty of Science operates on an annual budget exceeding $33 million and has approximately 200 full-time faculty members in eight departments: Applied Mathematics, Biology, Chemistry, Computer Science, Earth Sciences, Mathematics, Physics and Astronomy, and Statistical and Actuarial Sciences. The Faculty of Science also offers Honors programs, including the Bachelor of Medical Sciences, in collaboration with the basic science departments in the Faculty of Medicine and Dentistry. Nearly 3,900 full-time undergraduate students are registered in Science programs, and the Faculty enrols students from across the University as non-majors. The Faculty's outstanding programs annually attract an incoming class that is among the most highly qualified in the country. The Faculty is very active in research and graduate education, with over 425 full-time and 32 part-time graduate students. The Faculty of Science plays a central role in a number of major interdisciplinary research centres, including Surface Science Western, the Nanofabrication Facility, and Environmental Research Western, and offers strong interdisciplinary programs in Environmental Science and Genetics.

The Committee will begin its consideration of candidates on **October 1, 2005**. Nominations and applications should be submitted to: **Dr. Greg Moran, Provost and Vice-President (Academic), Room 115, Stevenson-Lawson Building, The University of Western Ontario, London, Ontario, N6A 5B8. Telephone (519) 661-3110; fax (519) 661-3676.** Applications should include a curriculum vitae and the names of at least three referees. For additional information on the Faculty, please visit its web site at: **http://www.uwo.ca/sci**

The University of Western Ontario is committed to Employment Equity and welcomes applications from all qualified women and men, including visible minorities, aboriginal people and persons with disabilities.

Great scientists don't just fall from the sky.



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Faculty Position in Systems' Neuroscience

Department of Neuroscience Baylor College of Medicine

As part of a major new initiative in Neuroscience, Baylor College of Medicine is recruiting outstanding tenure track faculty. The successful candidate for this position will have either the Ph.D. and/or M.D., several years of postdoctoral training, be an accomplished or promising young investigator in systems' neuroscience and have existing or strong potential for extramural research grant support. Candidates with a fundamental interest in the biological mechanisms of normal behavior or behavioral disorders and who utilize the tools of modern biologically based neuroscience research such as functional brain imaging, electrophysiology in alert preparations and/or innovative behavioral paradigms with animal models ranging from primates to small circuits are encouraged to apply.

Send *curriculum vitae*, personal statement with research interests/plans, and have at least three letters of reference sent to: **Michael J. Friedlander, Ph.D., Chair, Department of Neuroscience, One Baylor Plaza, Houston, Texas, 77030** by **August 1, 2005**. Applications and statement of research interest (but not letters of reference) may be submitted electronically to **friedlan@bcm.tmc.edu**.

Baylor College of Medicine is an Equal Opportunity/Affirmative Action and Equal Access Employer.

ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Professor / Assistant Professor (Tenure Track) of Multifunctional Materials

The Department of Materials Science of ETH Zurich invites applications from candidates with several years of experience in the synthesis/fabrication, and characterization of inorganic materials. This includes the synthesis and integration of micro- and nano-materials into complex systems that have useful responses to electrical, optical, magnetic, and/or mechanical functionalities. It is expected that close, collaborative relationships with other department members, both theoretical and experimental, in all materials classes will be established.

The professor will be expected to teach students of Materials Science at all levels, as well as to hold special courses for other disciplines (e.g. physics, electrical engineering, chemistry). Courses at Master level may be taught in English.

The appointment will be either at full professor or assistant professor level, depending on the applicant's qualifications and expertise.

Please submit your application together with a curriculum vitae, a list of publications, a list of research activities, and a research statement **to the President of ETH Zurich, Prof. Dr. O. Kuebler, ETH Zentrum, CH-8092 Zurich, no later than August 31, 2005**. With a view towards increasing the proportion of female professors, ETH Zurich specifically encourages female candidates to apply.

USDA ars Agricultural Research Service
www.ars.usda.gov

Research Biologist; Research Chemist (Biochemistry)

Western Regional Research Center

Salary Range of $68,530.00 - $125,193.00 PA

The USDA, Agricultural Research Service, is seeking a permanent, full-time scientist to conduct research at the Foodborne Contaminant Research Unit located in Albany, CA. The incumbent's assignment is to perform team research within a broad effort addressing the detection of foodborne toxins, including novel science and technology for assays and sampling strategies. The incumbent is responsible for developing 'in vitro' models for toxicity and conducting dosage studies with toxins in foods. Objectives include both fundamental and applied research on high impact topics.

For application directions, see **www.afm.ars.usda.gov/divisions/hrd/index.html** Announcement Number is **ARS-X5W-0298** This announcement closes **July 1, 2005**. For questions you may contact **Dr. John Mark Carter** at **510-559-6053** or e-mail: **mcarter@pw.usda.gov**.

USDA, ARS, is an Equal Opportunity Employer and Provider.

MRC Medical Research Council

MRC Laboratory of Molecular Biology, Cambridge

Group Leader positions

The MRC Laboratory of Molecular Biology wishes to recruit talented group leaders in any area of Molecular Biology. The Laboratory currently houses 60 independent groups, organised internally as four Divisions (see http://www.mrc-lmb.cam.ac.uk) with strong inter-Divisional collaborations. With the construction of a new research building (see http://www2.mrc-lmb.cam.ac.uk/newlmb.html), the Laboratory will have the opportunity to open up new directions in its research.

Applications are invited from any individual whose research is likely to make a major contribution to the goals of the Laboratory, which are to understand biological processes at the molecular level. Appointments of junior group leaders will be made at programme leader-track level in the first instance. More senior appointments to tenured positions will also be considered, depending on experience and achievements.

The Laboratory would especially, but not exclusively, like to attract applications in certain areas, specifically molecular and cellular neurobiology, computational and theoretical biology, electron cryo-microscopy and structural analyses of large assemblies or membrane proteins. There is a strong interest in expanding our work on neurodegeneration at a molecular level. Finally, there is scope for group leaders with interests in mammalian biology, especially haematopoiesis, immune function, cancer, development and aging.

Further information about the new positions and the future plans of the Laboratory are available at http://www2.mrc-lmb.cam.ac.uk/groupleaderinfo.html. The Laboratory is well provided with equipment to which all have access and there are excellent central services. Research expenses and support for a small group are provided, without the necessity to write grants. Administrative duties are minimal and no teaching is required. Salaries will be internationally competitive. MRC offers an attractive package of benefits. Crèche/nursery, sports and other facilities are available on site.

Applications should include a full CV, an outline of current and future research interests and the names and addresses of three professional referees who have agreed to be contacted. Enquiries are welcome at any time, but for this recruitment for which positions are immediately available, please reply by 15th August.

Please quote job reference LMB/505/15 and e-mail to recruit@mrc-lmb.cam.ac.uk. or post to Recruitment Office, MRC Centre, Hills Road, Cambridge, CB2 2QH.

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POSITIONS OPEN

FACULTY POSITION
Center for Pharmacogenetics
Department of Pharmaceutical Sciences
University of Pittsburgh

We are seeking to fill a tenure-track faculty position at the rank of **ASSISTANT** or **ASSOCIATE PROFESSOR.** The successful candidate should have a Ph.D., M.D., and/or Pharm.D. and appropriate postdoctoral training in the broadly defined areas of pharmacogenetics or pharmacogenomics. A strong research background with existing or the potential for NIH funding is necessary. The Center for Pharmacogenetics in the Department of Pharmaceutical Sciences is well equipped to perform state-of-the-art pharmacogenetic/pharmacogenomic research. There is excellent opportunity for interactions with other University of Pittsburgh and University of Pittsburgh Medical Center faculty. The Center is committed to excellence in extramural funded research and all current Center members have NIH funding. For four consecutive years, the School of Pharmacy has been ranked among the top 10 schools in the nation based on NIH funding. Existing research programs include genetic and pharmacological regulation of drug metabolizing/detoxifying enzymes and transporters, gene therapy and pharmacotherapy, molecular biology of protein degradation, and proteomics. For more information about the Center and Department, see **website: http://www.pharmacy.pitt.edu/Research/pharmgen.**

Applicants should submit a letter of interest, complete curriculum vitae, a one- to two-page description of future research, and the names of at least three references to: **Dr. Wen Xie, Search Committee Chair, Center for Pharmacogenetics, Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA 15261. E-mail: wex6@pitt.edu.** *The University of Pittsburgh is an Affirmative Action/Equal Opportunity Employer.*

FACULTY POSITIONS
University of Pennsylvania
School of Medicine

The Department of Psychiatry at the University of Pennsylvania School of Medicine is seeking highly qualified candidates for several faculty positions. The faculty appointments will be at either the **ASSISTANT, ASSOCIATE,** or **FULL PROFESSOR** rank and in the tenure track. Rank will be commensurate with experience. The successful applicants will have experience in the fields of neuroscience, neurobiology and behavior, addictions research, neuropharmacology, genetics, genetics of epilepsy, and mathematical genetics depending on the specific qualifications for each position. Applicants must have an M.D. or Ph.D., or M.D./Ph.D. degree. For a specific description of each faculty position, please see "Job Opportunities" at **website: http://www.med.upenn.edu/fapd/.** For information about the Psychiatry Department, please visit **website: http://www.med.upenn.edu/psychiatry.html.** Please submit curriculum vitae, a letter of interest, three reference letters, and indicate Job Reference Number to: **Dwight L. Evans, M.D., c/o Ava Plotnick, Department of Psychiatry, University of Pennsylvania School of Medicine, 305 Blockley Hall, 423 Guardian Drive, Philadelphia, PA 19104. E-mail: plotnick@mail.med.upenn.edu.**

The University of Pennsylvania is an Equal Opportunity/Affirmative Action Employer. Women and minority candidates are strongly encouraged to apply.

RESEARCH SCIENTIST/POSTDOCTORAL POSITION available at the University of Michigan for a highly motivated individual to study the mechanism of action of microsomal cytochrome P450 using biophysical methods and site-directed mutagenesis. Knowledge of the structure and function of heme and flavoproteins desirable. Individuals with expertise in cryoenzymology and rapid quench electron paramagnetic resonance will be considered favorably. Salary commensurate with experience. Send resume and names of three references to: **L. Waskell** at **e-mail: waskell@umich.edu.**

POSITIONS OPEN

POSITION ANNOUNCEMENT

Molecular Studies of Infectious Diseases: The Department of Veterinary Pathobiology, Center for Veterinary Health Sciences (CVHS), Oklahoma State University (OSU) invites applications for a tenure-track **RESEARCH FACULTY POSITION** in infectious diseases at the rank of **ASSISTANT** or **ASSOCIATE PROFESSOR.** Applications are encouraged from individuals with training/interests in bacteriology, virology, and/or immunology. Applicants should have a Ph.D. in molecular biology, microbiology, immunology, or biochemistry. Individuals with the D.V.M. or equivalent degree are encouraged to apply. Postdoctoral experience in molecular aspects of infectious diseases and/or immunity is preferred. Responsibilities include the development of a strong extramurally funded research program utilizing modern, molecular approaches to solving problems relating to animal or human diseases, and participation in the CVHS graduate education program. Opportunities exist for collaboration with other research faculty in the CVHS, Oklahoma Agricultural Experiment Station, University of Oklahoma Health Sciences Center and other OSU departments involved with molecular genetics and infectious disease research. Interested individuals should send an application including curriculum vitae, statement of professional goals, and names of three references to: **Anthony W. Confer, Head, Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Stillwater, OK 74078. Telephone: 405-744-6744.** To ensure full consideration, applications should be received by July 1, 2005, and review of applications will continue until a suitable candidate is identified.

RESEARCH POSITIONS

The University of Colorado Health Sciences Center Program in Urosciences has openings for three positions at the Postdoctoral level or above: one to help the Principal Investigator with studies of the structure and function of various signal transduction pathways in renal epithelial cells and a second position to assist with various signal transduction pathways in prostate cancer. A third position will help with studies on partially obstructed bladder and in bladder cancer. A Doctorate degree (Ph.D., M.D., or D.V.M.) with two or more years experience in molecular biology and or protein chemistry is required. Experience with protein purification and use of fast performance liquid chromatography and high performance liquid chromatography is expected for first position. Prior experience with animal model systems is a plus. Please send curriculum vitae and contact information of three references to: **Eric Brody, Assistant to Hari K. Koul, Professor and Director of Research, Division of Urology, The University of Colorado Health Sciences Center, 4200 East 9th Avenue, Box C319, Denver, CO 80262.** Or **fax: 303-315-1252** or **e-mail: eric.brody@uchsc.edu.**

The University of Colorado is committed to diversity and equality in education and employment.

POSTDOCTORAL POSITIONS: EPITHELIAL DIFFERENTIATION AND MEMBRANE BIOGENESIS

To study the structure and function of the highly specialized mammalian bladder urothelial membrane (**website: http://www.med.nyu.edu/sun/**). Contact:

Tung-Tien Sun, Ph.D.
Rudolf Baer
Professor of Dermatology
550 First Avenue
New York University Medical School
New York, NY 10016
E-mail: sunt01@med.nyu.edu
Fax: 212-263-8561

POSITIONS OPEN

RESEARCH ASSISTANT PROFESSOR. This position is a full-time nontenure-track position in the Department of Molecular and Cellular Biochemistry. The individual should hold a Ph.D. or equivalent degree in biochemistry or a related field and have at least three years of postdoctoral experience with peer-reviewed publications in respectable scientific journals. The successful candidate should have experience in conducting studies on the mammalian signal transduction pathways and the regulation of ion channel function. He/She should be familiar with cell culture techniques, cell transfection techniques, and molecular biological techniques. The successful candidate will be expected to obtain extramural funding to support their salary and their research. If offered this position, you will be required to pass a pre-employment drug screen as required by University of Kentucky Human Resources. Interested individuals should send their curriculum vitae and three references to:

Research Assistant Professor Search Committee
Department of Molecular and Cellular Biochemistry
MS607, University of Kentucky Chandler Medical Center
800 Rose Street
Lexington, KY 40536-0298

The University of Kentucky is an Equal Opportunity Employer and encourages applications from women and qualified minorities.

POSTDOCTORAL RESEARCH ASSOCIATE
Center for Neurodynamics
University of Missouri at St. Louis

A Postdoctoral position in experimental and computational neurophysiology is available in the Center for Neurodynamics at the University of Missouri at St. Louis. Research will involve in vivo imaging of neocortical seizures in the rat, using various imaging methods (intrinsic optical signal, calcium dye imaging, voltage sensitive dye imaging), as well as analysis of patterns of seizure spread using nonlinear-dynamics-based techniques. Research may also include computational studies of synchronization in neural systems. We are seeking a highly motivated scientist with expertise in at least two of the following areas: (1) experimental neuroscience and brain imaging, (2) experimental electrophysiology, (3) nonlinear dynamics in biological systems. A Ph.D. in physics, neuroscience, or related field is required. Salary will be competitive and commensurate with experience. Applications, including curriculum vitae, description of research interests, PDF files of representative publications, and the names of three references, should be sent to: **Dr. Sonya Bahar** at **e-mail: bahars@umsl.edu.** *The University of Missouri-St. Louis is an Affirmative Action/Equal Opportunity Employer committed to excellence through diversity.*

ASSISTANT PROFESSOR: The Department of Biomedical Sciences, Colorado State University seeks a tenure-track Assistant Professor. Expertise in any aspect of mammalian reproductive biology, including stem cell/developmental, vascular, or pathologic aspects of reproductive biology, is sought. The candidate will be expected to use state-of-the-art cellular and molecular approaches to develop an extramurally funded research program, and to participate in the educational activities of the Department. A description of this position and research interests of faculty can be found at **website: http://www.cvmbs.colostate.edu/bms.** Applicants must have postdoctoral research experience, submit a letter of application, curriculum vitae, statements of research and teaching interests, and list of three references to: **Dr. R. V. Anthony, ARBL-Campus Delivery 1683, Colorado State University, Fort Collins, CO 80523-1683. E-mail: russ.anthony@colostate.edu.** Application review will begin August 15, 2005, and continue until a suitable candidate is found.

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Industry Recruiter

Mr. Jensen has over 20 years of experience in human resource consulting and staffing for the biotechnology and pharmaceuticals industry.

Adviser Bill Lindstaedt
Director,
UCSF Career Center

Mr. Lindstaedt has been providing career related advice to scientists and engineers for nearly 15 years, with a particular emphasis on working with graduate-level trainees in the life sciences.

Adviser Naledi Saul
Assistant Director,
UCSF Career Center

Ms. Saul has 7 years of career counseling with 4 years focused on counseling graduate students and postdocs in the biomedical and health sciences. Her forte is working with scientists pursuing careers in the public health arena.

Adviser Jim Austin
Editor, Science's
Next Wave

Dr. Austin has a Ph.D. in physics and worked in academia before coming on board to write about traditional and nontraditional career paths for scientists.

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POSITIONS OPEN

POSTDOCTORAL POSITION

Postdoctoral position is available to study cell and molecular and biology of complications of diabetes mellitus and kidney development. Potential candidates must have documented experience of one or more years in cell and molecular biology techniques. Please send curriculum vitae with three references to:

Yashpal S. Kanwar, M.D., Ph.D.
Northwestern University Medical School
303 E. Chicago Avenue
Chicago, IL 60611 U.S.A.
E-mail: y-kanwar@northwestern.edu

PROFESSOR OF IMMUNOPATHOLOGY
Department of Pathology
Harvard Medical School

The Department of Pathology at Harvard Medical School is seeking to fill the Edward Mallinckrodt, Jr. Professor of Immunopathology. Candidates should be dynamic scientific leaders in immunology as demonstrated by their scientific accomplishments and international reputation. The successful candidate is expected to participate in the training of graduate students enrolled in the Committee on Immunology, the Program in Biological and Biomedical Sciences, and the medical student teaching programs of the Department of Pathology. The candidate is expected to direct a vigorous research program, and recently constructed laboratory space is available within the Department of Pathology at Harvard Medical School in the New Research Building.

Candidates should send curriculum vitae and statement of research interests to:

Mallinckrodt Search Committee
c/o Dr. Peter M. Howley
Harvard Medical School
77 Avenue Louis Pasteur
NRB, Room 950
Boston, MA 02115

Harvard Medical School is an Affirmative Action/Equal Opportunity Employer. Qualified female and minority applicants are encouraged to apply.

TROPICAL FOREST BIOLOGIST
Arnold Arboretum of Harvard University

The Arnold Arboretum seeks a tropical forest biologist to: (1) assist the Scientific Director of the Center for Tropical Forest Science-Arnold Arboretum Asia program through forest plot research, training for forest scientists, and new plot establishment; (2) research in forest ecology and systematics; (3) undergraduate instruction. Requires: (1) Ph.D. and record of research accomplishments; (2) ten years field experience; (3) record of successful teaching; (4) software and database development skills; (5) fluency in the taxonomy of Southeast Asian flora. Send letter of application, resume, and contact information for three references to: **Dr. Robert Cook, Arnold Arboretum, 125 Arborway, Boston, MA 02130** or apply at **website: http://www.harvard.edu/jobs/.** *The Arnold Arboretum of Harvard University is an Equal Opportunity/Affirmative Action Employer.*

The University of Florida Genetics Institute seeks an outstanding candidate to fill a **TENURE-TRACK BIOINFORMATICS FUNCTIONAL GENOMICS POSITION.** Academic rank and departmental affiliation will be determined based on the successful applicant's qualifications and specific research interests. Review of applications will begin immediately and will continue until the application deadline of July 31, 2005. Please send curriculum vitae, statement of research interest/plans, and names of three references to: **Nicholas Muzyczka, Ph.D., Bioinformatics/Functional Genomics Search Committee, University of Florida Genetics Institute, P.O. Box 100196, Gainesville, FL 32610-0196.**

Reference position # 00021321 when applying.

FACULTY POSITION
INFECTIOUS DISEASES
University of Maryland, College Park

The Virginia-Maryland Regional College of Veterinary Medicine at the University of Maryland in College Park, Maryland, invites applications from qualified individuals for a tenured/tenure-track faculty position in infectious disease research. This appointment will be at the **ASSISTANT/ASSOCIATE PROFESSOR** level, rank and salary commensurate with training and experience. A D.V.M./Ph.D. or Ph.D. degree with relevant postdoctoral training is required. Current department focus is on host-pathogen interaction with emphases in virology, immunology, bacterial pathogenesis, epidemiology, and public health. Investigators with research emphases in animal, zoonotic, and/or public health diseases are encouraged to apply. The position requires 80 percent research and 20 percent teaching responsibilities, to include active involvement in the Virginia-Maryland Search Committee Graduate Program. The successful candidate will be expected to develop, maintain, and conduct a productive, extramurally funded research program that will strengthen the current research goals of the College. Submit a letter of application, curriculum vitae, statement of career goals, and names of three professional references to: **Dr. Nathaniel L. Tablante, Jr., Search Committee Chair, VA-MD Regional College of Veterinary Medicine, Maryland Campus, 8075 Greenmead Drive, University of Maryland, College Park, MD 20742-3711. Fax: 301-314-6855; e-mail: nlt@umd.edu.** Applications will be accepted until July 31, 2005, or until a suitable candidate is identified. The position is available immediately. *The University of Maryland is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply.*

The Department of Microbiology, Immunology, and Parasitology at Louisiana State University Health Sciences Center (LSUHSC) in New Orleans is seeking a researcher for a tenure-track position at the **ASSISTANT/ASSOCIATE PROFESSOR** level who has demonstrated excellence in the general area of cancer virology. Research with potential clinical translation is encouraged. This position will be supported jointly with the Stanley S. Scott Cancer Center. External National Cancer Institute grants are required at the Associate Professor level. Please send curriculum vitae and the names of three references to: **Ronald Luftig, Chair of the Search Committee, Microbiology, Immunology, and Parasitology, 1901 Perdido Street, Box P6-1, New Orleans, LA 70112-1393.** *LSUHSC is an Equal Employment Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITION

Brown Medical School, Division of Endocrinology, Hallett Center for Diabetes and Endocrinology, Rhode Island Hospital. Opportunity to study the molecular mechanisms of obesity-related insulin resistance and type 2 diabetes as well as adipose energy metabolism. Fully funded for three years. Ph.D. and/or M.D. plus strong background in molecular biology and mammalian cell culture required; background in obesity and diabetes as well as experience in the study of metabolism in vivo desirable but not necessary.

Please send curriculum vitae (including reference contact information) to: **Jane Conti-Dutko, 1 Hoppin Street, Suite 200, Providence, RI 02903.**

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U.S. Department of Health and Human Services

Website: http://www.training.nih.gov

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POSTDOCTORAL POSITIONS
Pathology and Laboratory Medicine
Emory University

Two highly motivated individuals are wanted for Postdoctoral positions at Emory University School of Medicine, Atlanta, Georgia, the United States. Strong molecular biology training is required. These two positions are available immediately and the salary is dependent on the experience.

My laboratory is interested in nerve growth factor signaling in neuronal cell survival. Moreover, we are also interested in phosphatidylinositol 3-kinase enhancer (PIKE) guanosine triphosphate's role in a variety of brain cancers. Future available research projects include: (a) the role of nuclear proteins in preventing apoptosis; (b) the function of PIKE in the brain tumor; (c) PIKE's role in metabotropic glutamate receptor/AMPA receptors crosstalk; (d) PIKE knockout and its phenotype study.

For reference, please read recent papers: (1) *Nature Neuroscience* **6:**1153–1161, 2003; (2) *JBC* **279:**16441–51, 2004; (3) *Proc. Natl. Acad. Sci.* **101:**6993-8, 2004; (4) *Oncogene* **23:**8447–8454, 2004; (5) *EMBO J.* **23 (20):**3995–4006, 2004; (6) *Proc. Natl. Acad. Sci.* **101:**18200–8205, 2004.

If interested, please contact: **Dr. Keqiang Ye** at **telephone: 404-712-2814** or **e-mail: kye@emory.edu.**

Emory University is an Equal Opportunity/Affirmative Action Employer.

National University of Singapore
Department of Chemical and Biomolecular Engineering

The Department of Chemical and Biomolecular Engineering at National University of Singapore invites applications for tenure-track **FACULTY POSITIONS** at all levels. The Department is one of the largest internationally with excellent in-house infrastructure for experimental and computational research. A Ph.D. in chemical engineering or related areas and a strong research record with excellent publications are required. Please refer to **website: http://www.chbe.nus.edu.sg/** for more information on the areas of interest and for application details. Applicants should send full curriculum vitae (including key publications), a detailed research plan, a statement of teaching interest, and a list of names of at least three references to: **Professor Raj Rajagopalan, Head of Department (Attention: Ms. Nancy Chia, e-mail: checls@nus.edu.sg).**

The Department of Microbiology, Immunology, and Parasitology at Louisiana State University Health Sciences Center (LSUHSC) invites applications for a tenure-track position at the **ASSOCIATE PROFESSOR** level for an outstanding candidate with a strong interest in infectious diseases. Successful candidate will be an active member of the Stanley S. Scott Cancer Center. The Department includes 15 funded faculty members actively involved in host-pathogen research. Applications should have a Ph.D. or equivalent degree with postdoctoral experience and an established, funded research program. Send curriculum vitae, statement of research interests, and three letters of reference to: **Dr. Ronald B. Luftig, Chair of the Search Committee, Microbiology, Immunology, and Parasitology, 1901 Perdido Street, Box P6-1, New Orleans, LA 70112-1393.** *LSUHSC is an Equal Employment Opportunity/Affirmative Action Employer.*

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Contact: Carol Hamel, Admissions Coordinator, admissions@mbl.edu, (508) 289-7401, 7 MBL Street, Woods Hole, MA 02543

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Symposium – 8:15 a.m. to 6:30 p.m.

To register for the Symposium please visit:
www.childrenshospitalla.org/symposiumregistration

S P E A K E R S

Sir Magdi Yacoub, FRS, FRCS, FRCP, DS
Heart Science Centre, Harefield Hospital, Harefield, UK

Professor Martin Pera
Monash University and the Australian Stem Cell Centre Clayton, Victoria, Australia

Peter Lansdorp, MD, PhD
University of British Columbia, Vancouver, BC, Canada

Margaret Goodell, PhD
Baylor College of Medicine, Houston, TX

Eli Keshet, PhD
Hebrew University - Hadassah Medical School, Jerusalem, Israel

Vincent Fleury, PhD
Groupe Matière Condensée et Matériaux, Groupe Biophysique, Université de Rennes

Paul Sternberg, PhD
California Institute of Technology, Pasadena, CA

Anthony Atala, MD
Wake Forest University, Winston-Salem, NC

Gail Naughton, PhD
San Diego State University, San Diego, CA

Peter Zilla, MD, PhD
University of Cape Town, Cape Town, South Africa

James B. Bassingthwaighte, MD, PhD
University of Washington, Seattle, WA

David Williams, DSc FREng
UK Centre for Tissue Engineering, Liverpool, UK

2006 Career Awards in the Biomedical Sciences

Deadline: October 3, 2005

$500,000 award over five years for postdoctoral fellows

- These awards provide $500,000 over five years to support up to two years of advanced postdoctoral training and the first three years of a faculty appointment
- Candidates must be citizens or permanent residents of the U.S. or Canada at the time of application
- BWF will make at least 20 awards in 2006
- U.S. or Canadian degree-granting institutions must submit nominations on behalf of individual candidates
- Approximately half the awards go to researchers with a Ph.D. degree and half to those with an M.D. or M.D.-Ph.D. degree
- Complete program information, eligibility guidelines, and application forms are available on BWF's website at www.bwfund.org

2005 Career Award Recipients (22 awards)

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University of California-San Francisco

Ben E. Black, Ph.D.
University of California-San Diego School of Medicine

David L. Brody, M.D., Ph.D.
Washington University School of Medicine

Mark M. Churchland, Ph.D.
Stanford University

William M. Clemons, Ph.D.
Harvard Medical School

Daniela M. Dinulescu, Ph.D.
Massachusetts Institute of Technology

Alla Y. Karpova, Ph.D.
Cold Spring Harbor Laboratory

Leslie S. Kean, M.D., Ph.D.
Emory University School of Medicine

Tobias R. Kollmann, M.D., Ph.D.
University of Washington School of Medicine

Steven T. Kosak, Ph.D.
Fred Hutchinson Cancer Research Center

Yaping J. Liao, M.D., Ph.D.
Stanford University School of Medicine

Feroz R. Papa, M.D., Ph.D.
University of California-San Francisco School of Medicine

Michael G. Poirier, Ph.D.
Northwestern University

Jeremy F. Reiter, M.D., Ph.D.
University of California-San Francisco

Kyu Y. Rhee, M.D., Ph.D.
Weill Medical College of Cornell University

Alan Saghatelian, Ph.D.
Scripps Research Institute

Annette E. Salmeen, D.Phil.
Stanford University School of Medicine

Michael D. Shapiro, Ph.D.
Stanford University School of Medicine

Helen C. Su, M.D., Ph.D.
National Institute of Allergy and Infectious Diseases (NIAID)

Sinisa Urban, Ph.D.
Harvard Medical School

Loren D. Walensky, M.D., Ph.D.
Harvard Medical School

Hong Zhang, Ph.D.
Harvard Medical School

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